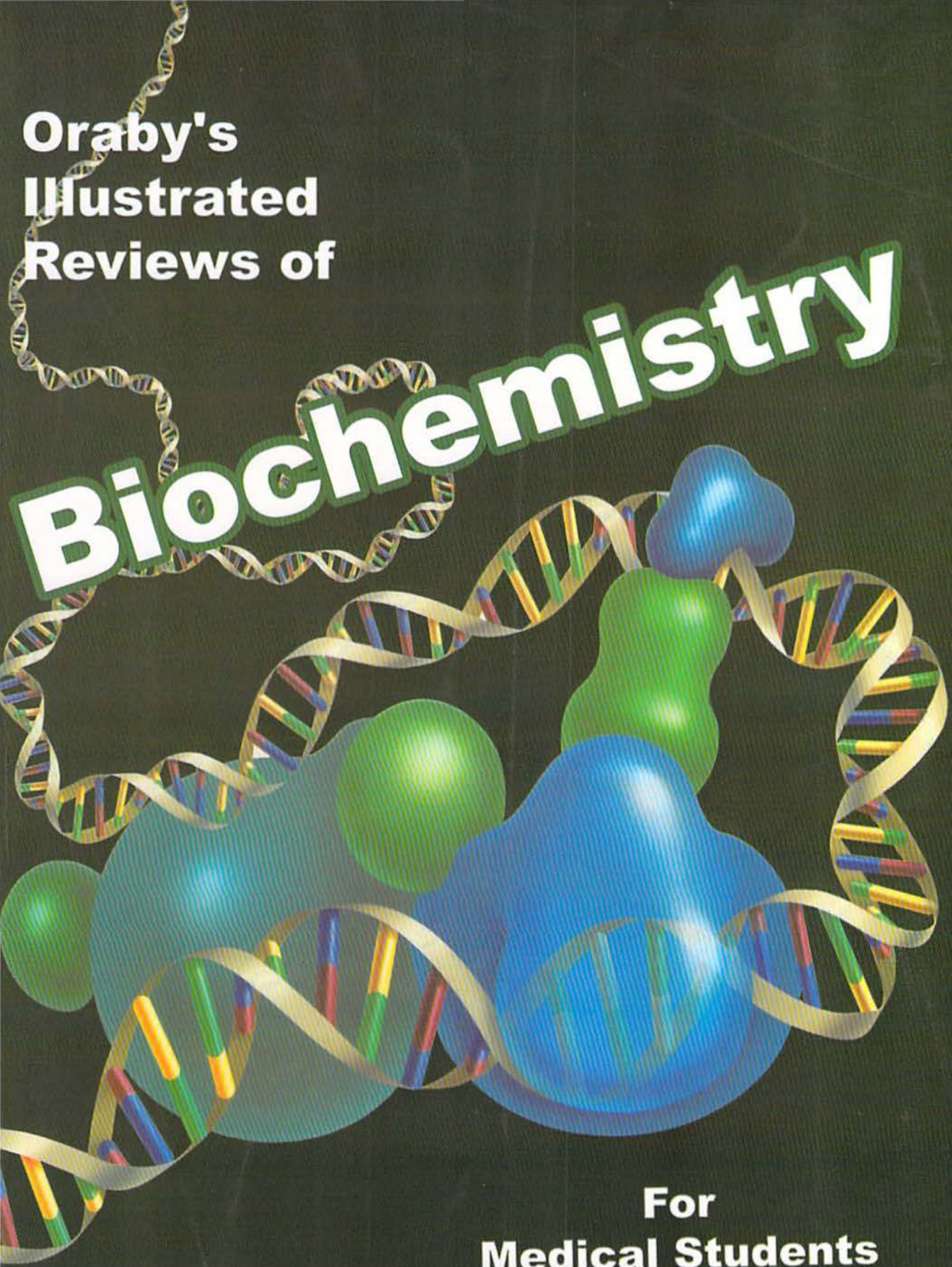
Oraby's Illustrated Reviews of Biochemistry



For
Medical Students
and Postgraduates
Part III

Oraby's Illustrated Review of

Biochemistry

For Medical Students
And Postgraduates

By

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(III)

Twelfth Edition

DEDICATION

To the memory of my mother who gave me every thing, and I missed her very much.

Note:
Dear student / colleague:
If you have any comment about this edition or further editions, please *mail your suggestions to*: m_s_oraby@hotmail.com

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Chapter 1 *Proteins Metabolism*

I. *Introduction:*

A. Proteins are organic compounds formed of α-amino acids joined together by peptide bonds.

B. In contrast to carbohydrate and lipids whose major function is to provide energy, the primary role of amino acids is to serve as building blocks for the synthesis of body proteins.

C. The amount of proteins present in the body of an adult man weighing 70 kg is about **12 kg.**

D. **The main functions of body proteins are:**

1. Synthesis of tissue proteins e.g. contractile protein of muscles, collagen. ...etc.
2. Synthesis of cell organelles as cell membrane, receptors ...etc.
3. Synthesis of enzymes.
4. Synthesis of milk proteins.
5. Synthesis of protein hormones.
6. Synthesis of plasma proteins.
7. Synthesis of nucleoproteins.

Proteins Digestion

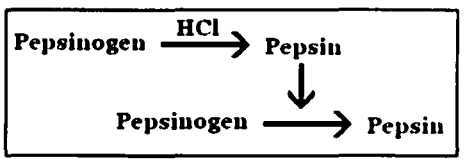
I. Proteins are too large to be absorbed by the intestine. Therefore, they must be hydrolyzed at first to give amino acids, which can be easily absorbed.

II. Three organs produce the enzymes needed for protein digestion: stomach, pancreas and small intestine.

III. ***In the stomach:*** The following enzymes start the digestion of proteins: pepsin, rennin and gelatinase.

A. **Pepsin (optimum pH: 1-2):**

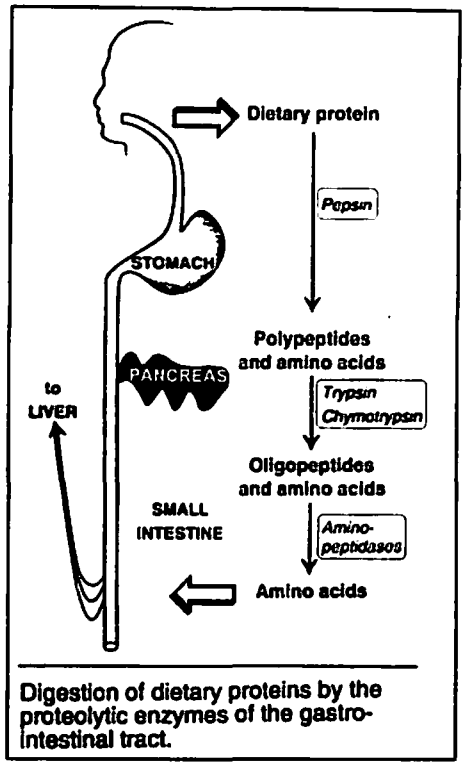
1. It is secreted by the body chief cells (serous cells) of the stomach as an inactive proenzyme: pepsinogen.
2. **Pepsinogen** is activated to pepsin at first by gastric HCl , then by auto catalysis by other pepsin molecules that have already been activated by HCl.



3. Action of pepsin:

a) Pepsin is endopeptidase i.e. acts on the amino acids in the middle of polypeptide chains.

b) It hydrolyses the bonds formed by aromatic amino acids e.g. tyrosine.



Digestion of dietary proteins by the proteolytic enzymes of the gastro-intestinal tract.

B. **Rennin (optimum pH: 4):**

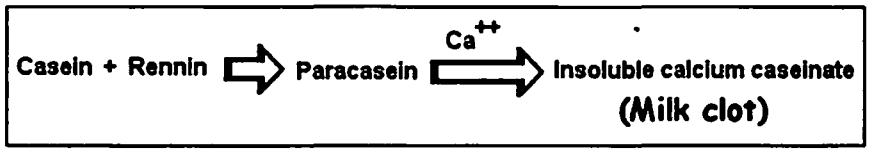
1. Action of rennin:

a) Rennin causes coagulation of milk proteins.

b) Rennin acts on casein, which is the main milk protein.

c) In presence of calcium ions, rennin converts casein into insoluble calcium caseinate (milk clot).

d) The digestion of calcium caseinate is completed by pepsin.



2. Rennin is absent in adults and present only in infant stomach.

3. Rennin is important for infant because the formation of milk clot prevents the rapid passage of the milk from the stomach. This gives the baby the **sense of fullness.**

C. **Gelatinase:** An enzyme liquefies gelatin.

IV. *In the intestine:*

Two organs, pancreas and intestine produce enzymes act on proteins in the intestine.

A. **Pancreatic enzymes:** These include trypsin, chymotrypsin, carboxypeptidase, elastase and collagenase.

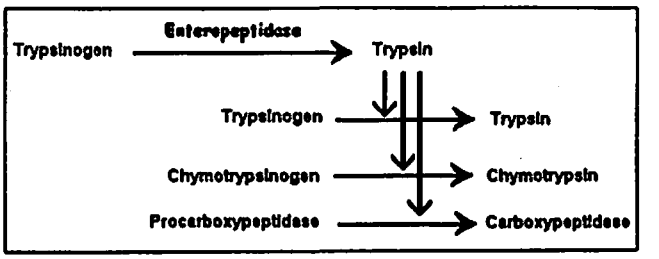
1. Trypsin (optimum pH: 8):

a) Trypsin is secreted as an inactive proenzyme: trypsinogen.

b) **Trypsinogen** is activated to trypsin at first by enteropeptidase enzyme (produced by intestinal mucosa), then auto catalytically by other trypsin molecules that have already been activated by enteropeptidase enzyme.

c) **Action of trypsin:**

1) Trypsin is an endopeptidase, hydrolyzing the peptide bonds formed by basic amino acids e.g. lysine and arginine. It releases smaller peptides and more free amino acids.



2) Trypsin acts also as activator for all other inactive pancreatic enzymes.

2. **Chymotrypsin:**

a) Chymotrypsin is secreted as inactive proenzyme; chymotrypsinogen. It is activated by trypsin enzyme.

b) ***Action of chymotrypsin:***

It is an endopeptidase, hydrolyzing the peptide bonds formed by aromatic amino acids (its action is similar to that of pepsin).

3. **Carboxypeptidase:**

a) It is secreted as an inactive proenzyme: procarboxypeptidase and activated by trypsin enzyme.

b) ***Action of carboxypeptidase:***

1) It is an exopeptidase i.e. acts on the periphery of polypeptide chains.

2) Carboxypeptidase hydrolyses the peptide bonds adjacent to the free -COOH group of the polypeptide chain, releasing each time a single free amino acid.

4. **Elastase:**

a) It is secreted as an inactive proenzyme: proelastase, and activated also by trypsin.

b) Action of elastase:

1) It is an endopeptidase.

2) In spite of its name, it hydrolyses the peptide bonds formed by small amino acids e.g. alanine, glycine and serine.

5. **Collagenase:** An enzyme that catalyses the hydrolysis of collagen.

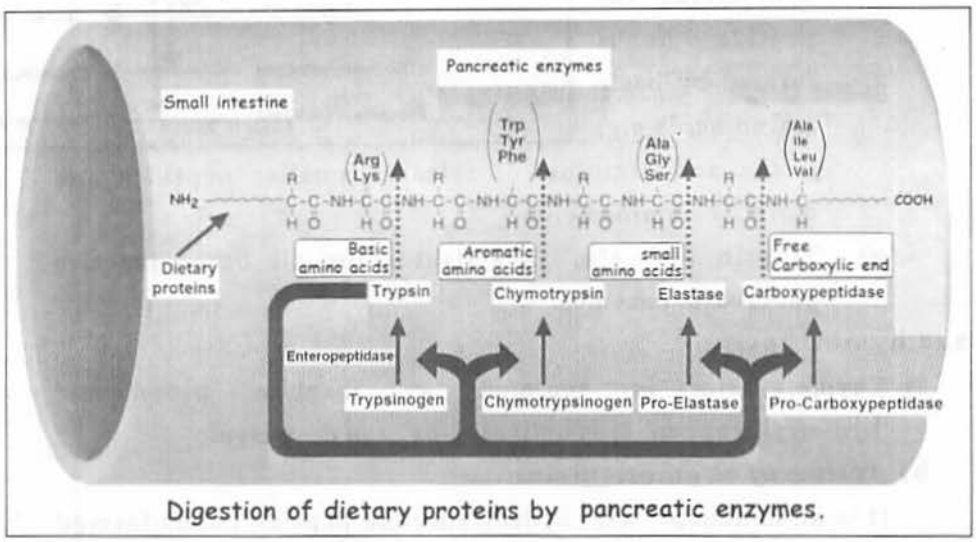
B. **Intestinal enzymes:**

1. **Aminopeptidase:**

a) It is an exopeptidase, hydrolyses the peptide bonds adjacent to the free -NH_2 group of the polypeptide chain, releasing each time a single free amino acid.

2. **Dipeptidase:**

a) It completes the digestion of dipeptides.



Digestion of dietary proteins by pancreatic enzymes.

V. *Gastrointestinal hormones that help protein digestion:*

A. **Gastrin:**

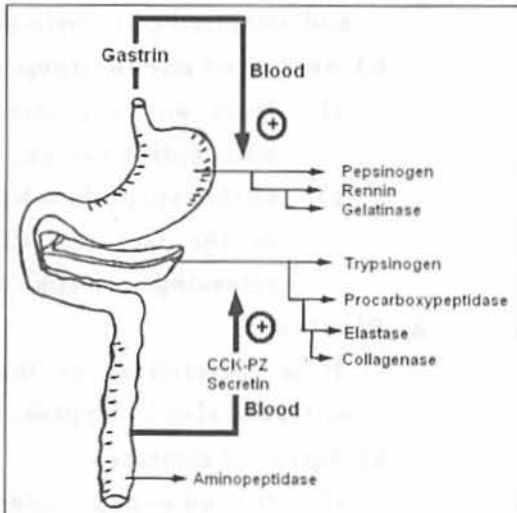
1. **Location:** Gastric antrum and duodenum.
2. **Major action:**
   a) Pepsinogen secretion.
   b) Release intrinsic factor from gastric mucosa.

B. **Cholecystokinin (CCK):**

1. **Location:** Duodenum and jejunum.
2. **Major action:** Pancreatic proenzymes secretion.

C. **Secretin:**

1. **Location:** Duodenum and jejunum.
2. **Major action:**
   a) Pancreatic proenzymes secretion.
   b) Pancreatic bicarbonate secretion.



Enzymes of protein digestion and hormones that stimulate their secretion

Protein Absorption

I. Introduction:

A. Under normal conditions, the dietary proteins are almost completely digested into amino acids.

B. These amino acids are then rapidly absorbed from small intestine into the portal circulation.

C. **In neolyborn infants proteins may be absorbed as such:**
Where gamma globulins present in colostrum are absorbed which give immunity to the baby.
(Colostrum is the milk secreted from mammary gland in the first few days after labor).

II. Site of absorption:

Small intestine: jejunum and ileum.

III. Mechanism of amino acid absorption:

Amino acids are absorbed either by active transport mechanism where energy is required (L-amino acids) or by passive transport mechanism, which needs no energy (D-amino acids).

A. **Active transport:** for L-amino acids:
Two mechanisms are involved; carrier protein transport system and glutathione transport system.

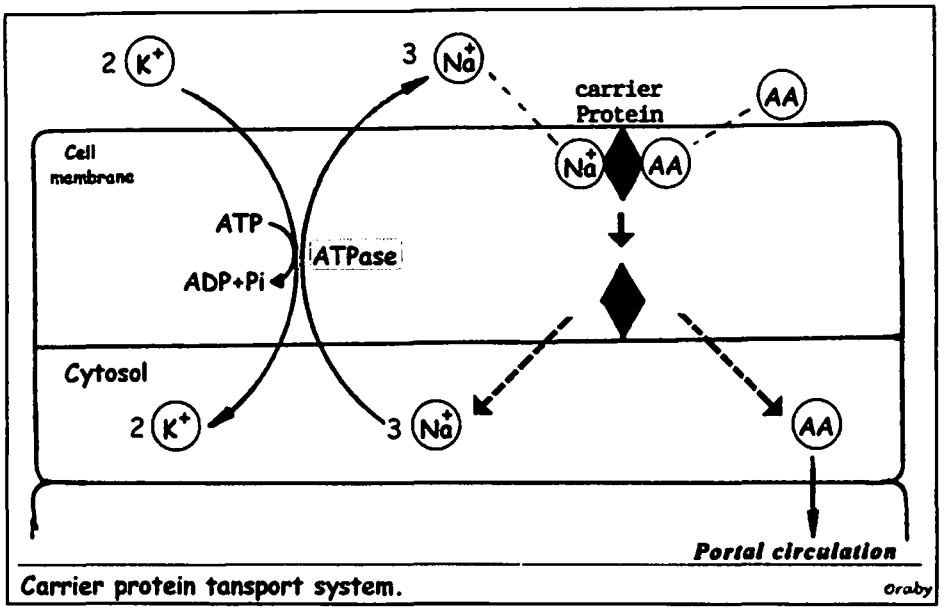
1. Carrier protein transport system:

a) Energy required is derived from sodium pump. For each amino acid one ATP molecule is utilized.

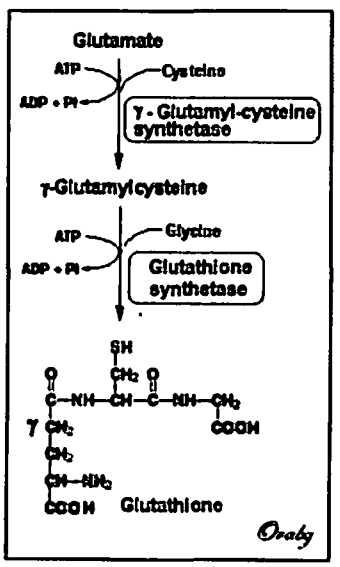
b) Amino acids are absorbed by specific carrier protein present in small intestine, by a mechanism similar to that of glucose absorption i.e. the carrier has one site for the amino acid and another site for sodium (see carbohydrate absorption, part II).

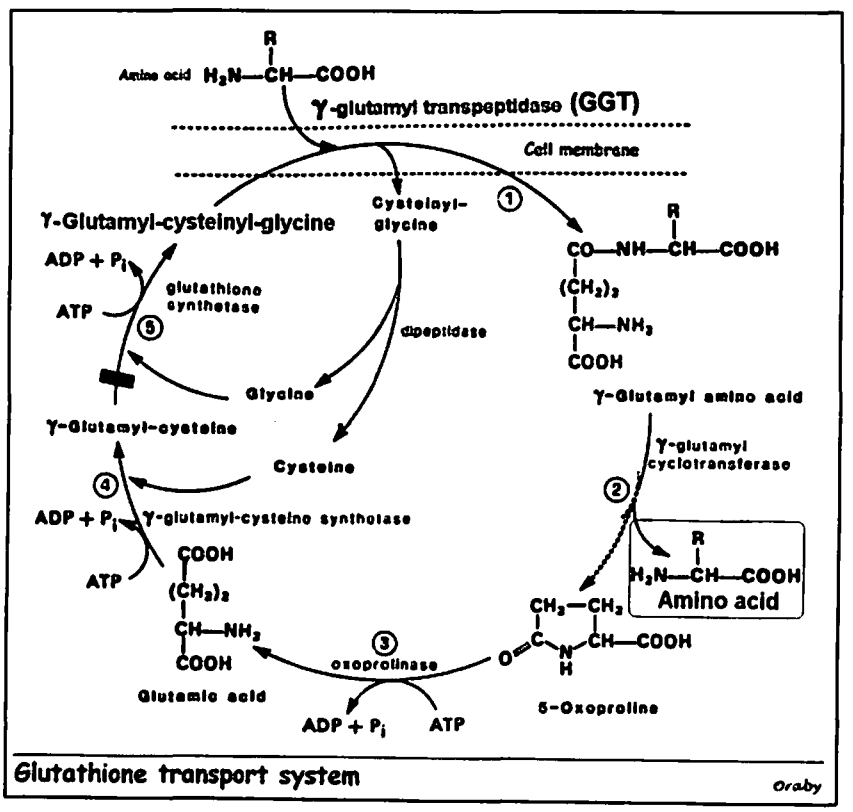
c) There are **five different amino acid carrier systems.** Each transports a group of closely related amino acids. These groups are:

1) Small neutral amino acids.
2) Large neutral amino acids.
3) Basic amino acids.
4) Acidic amino acids.
5) Imino acids.



2. **Glutathione transport system (γ-glutamyl cycle):**
   This transport system is for the uptake of amino acids from the intestine, kidney, brain and liver (bile ductule cells).





a) The amino acids react with glutathione (γ-glutamyl-cysteinyl-glycine) at the surface of the cell to form dipeptide that is transported across the membrane into the cytoplasm of the cell. This reaction is catalyzed by γ-glutamyl transpeptidase (transferase) enzyme (GGT).

b) The subsequent reactions of the cycle serve to regenerate glutathione.

c) 3 ATP molecules are utilized in these reactions.

d) **Functions of γ-glutamyl cycle:**

1) Transportation of amino acids from the extracellular space into the cytoplasm.

2) Synthesis of glutathione, which is an important reducing substance in the cells (see glycine metabolism).

e) **Diagnostic importance of GGT:** The blood concentration of this enzyme is elevated in:

1) **Cholestasis:** impairment of bile flow.

2) **Chronic alcoholism.**

Oxoprolinuria:

- *Is an inherited disease in which elevated levels of 5-oxoproline occur in blood and urine.*
- *It is due to deficiency of the glutathione synthetase enzyme.*
- *It causes acidosis and neurological damage.*

B. **Passive transport:**

D-Amino acids are absorbed by simple diffusion i.e. needs no energy.

Plasma amino acids

I. *Introduction:*

A. The plasma level of most amino acids does not remain constant throughout the 24 hours of the day i.e. there is a circadian changes.

B. It varies from 4 to 8 mg / dl plasma.

C. The level depends on the nutritional state whether it is post absorption or fed state.

II. *Post-absorption (fasting) state:* i.e. 12 hours after last meal:

A. The plasma amino acids are tending to be decreased.

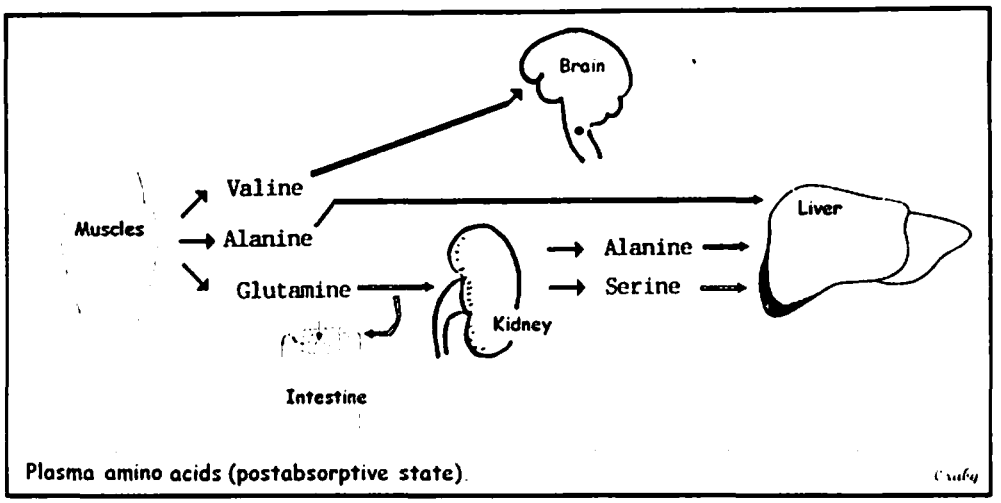
B. Amino acids are released from endogenous protein stores mainly muscles and kidney:

1. **Muscles: provide** mainly alanine, glutamine and valine.
2. **Kidneys: provide** alanine and serine.

C. **The released amino acids are taken up by:**

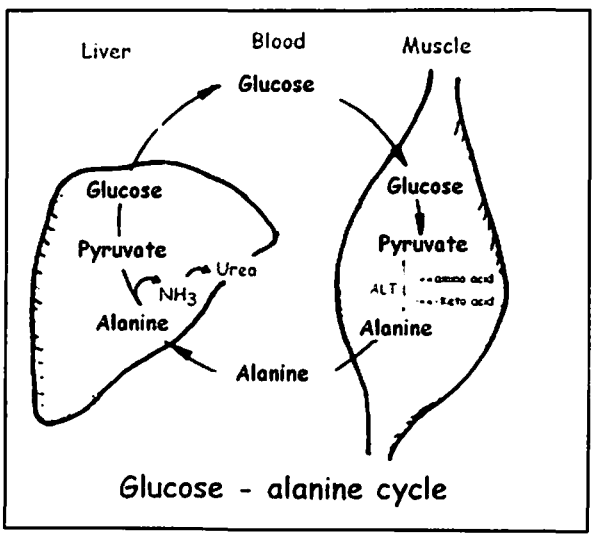
1. **Brain:** valine.
2. **Gut and kidney:** Glutamine, which is converted into ammonia.

3. **Liver:** Serine and alanine: Alanine is the major glycogenic amino acid that can be converted into glucose.



D. Glucose-alanine-cycle:

1. Alanine is synthesized in muscle by transamination of pyruvate and released into blood stream.
2. Liver then takes up alanine where it is converted to glucose (Gluconeogenesis).
3. Glucose is released into the blood stream where it can be taken up by muscles and used for synthesis of alanine.



III. *Fed state (after meal):*

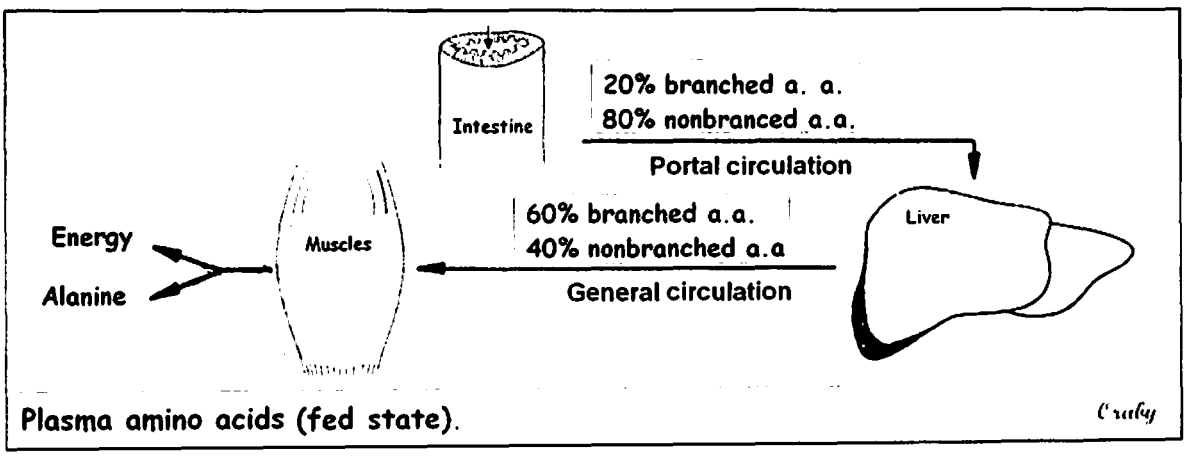
A. The amino acids present in diet contain about 20% branched chain amino acids and 80% of non-branched amino acids.

B. After the ingestion of a protein rich meal, amino acids are absorbed from the gut via portal circulation to the liver.

1. **Liver** utilizes most non branched amino acids releasing into systemic circulation the remaining branched amino acids. Thus branched amino acids constitute about 60% of the plasma amino acids.
2. **Muscles** extract the amino acids (mainly the branched chain) where they undergo:

a) Oxidation for energy production.

b) Conversion into alanine where again may give glucose in the liver.



Plasma amino acids (fed state).

Amino acid pool

I. *Introduction:*

A. Free amino acids distributed throughout the body are about 100 grams and they are called amino acid pool.

B. Of these 100 grams, about 50% is in the form of glutamate and glutamine.

II. *Sources and fate of Amino acid pool:*

A. Sources of amino acid pool:

1. Dietary proteins (absorbed amino acids).
2. Hydrolysis of body proteins.
3. Synthesis of non-essential amino acids.

B. Fate of amino acid pool:

1. Anabolic pathways:

a) Synthesis of proteins: tissue proteins, enzymes, hormones etc.

b) Synthesis of specialized products. Each amino acid enters in the structure of some products special for it e.g. glycine enters in the structure of creatine, heme...etc.

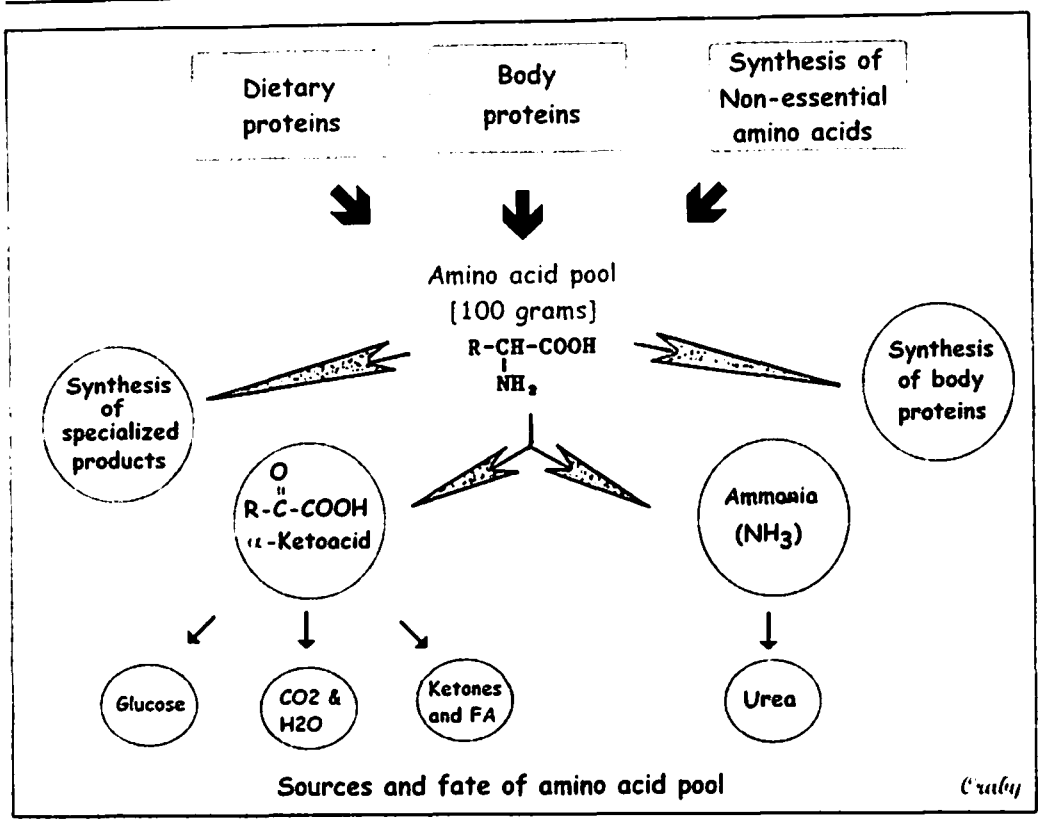
c) Synthesis of small peptides e.g. glutathione.

2. Catabolic pathways:

a) Removal of amino group (NH_2) from amino acids by transamination and deamination processes.

b) The resulting products are ammonia, urea and α-keto acids.

c) α-Keto acids are further metabolized to be completely oxidized into CO_2, H_2O or may be converted into glucose or fatty acids and ketones.



General catabolic pathways of amino acids

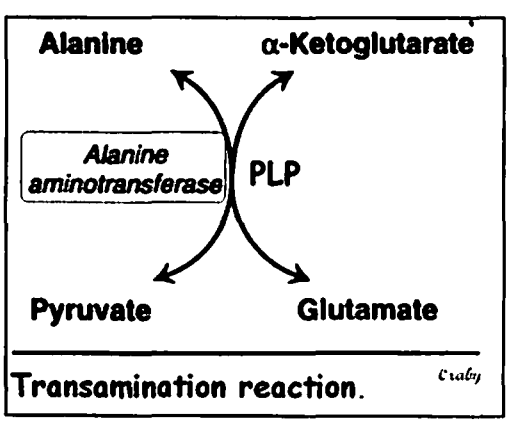
In human, the end products of protein and amino acids catabolism are ammonia and urea. They are produced through the following catabolic pathways:

- ***Transamination.***
- ***Deamination:*** *Oxidative-Non oxidative-Hydrolytic.*
- ***Transdeamination:*** ***i.e.****transamination followed by deamination.*
- ***Decarboxylation.***

I. *Transamination:*

A. Definition:

It is the transfer of amino group from α-amino acid to α-ketoacid to form a new α-amino acid and a new α-ketoacid.

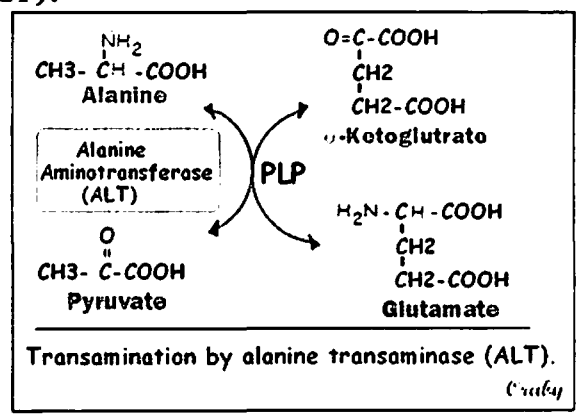


B. Mechanism:

1. Enzymes called transaminases (or amino transferases) catalyze transamination reactions.
2. Pyridoxal phosphate (PLP = active vitamin B_6) is the coenzyme of all transaminases.
3. All amino acids (except lysine, threonine, proline and hydroxyproline) may undergo transamination.
4. All transamination reactions are reversible.
5. All transaminases are present either in cytosol or in both cytosol and mitochondria of most tissues.
6. Among all transaminases, 3 are present in most mammalian tissues and they are of clinical importance. These are: ALT , AST and glutamate transaminase .

a) Alanine transaminase (ALT):

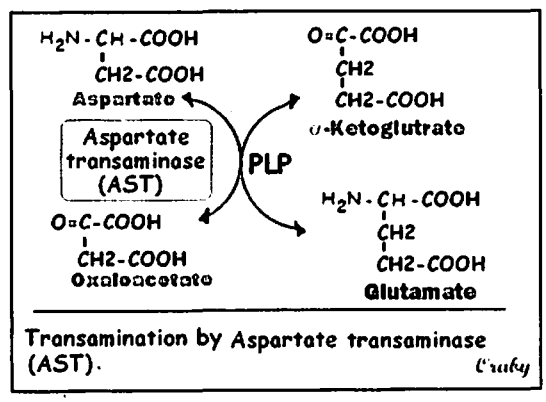
1) ALT also called glutamate pyruvate transaminase **(GPT).**
2) ALT is present in the cytosol of the cells.
3) ALT is an enzyme that catalyses the transfer of amino group from alanine to α-ketoglutarate to form glutamate and pyruvate. The reaction is reversible.



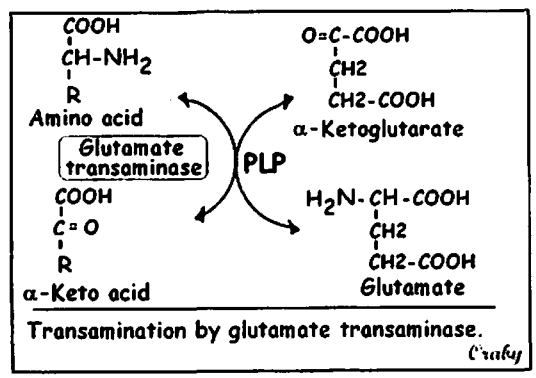
Transamination by alanine transaminase (ALT).

b) Aspartate transaminase (AST):

1) AST is also called glutamate oxaloacetate transaminase **(GOT).**
2) AST is present in both cytosol and mitochondria of the cells.
3) AST is an enzyme that catalyses the transfer of amino group from aspartate to α - ketoglutarate to form glutamate and oxaloacetate. The reaction is reversible.



Transamination by Aspartate transaminase (AST).



Transamination by glutamate transaminase.

c) Glutamate transaminase:

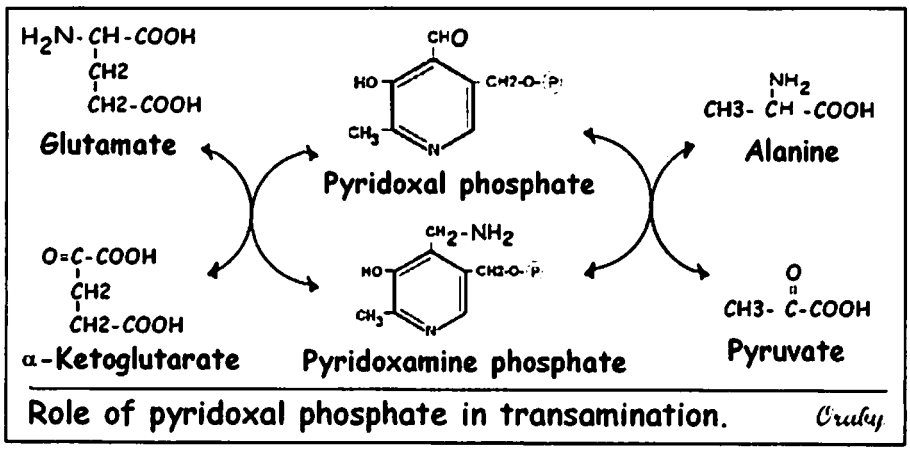
1) This enzyme catalyses the transfer of amino group from any amino acid to α-ketoglutarate.

C. Functions of transamination:

1. Transfer of amino group ($-NH_2$) from most amino acids to α-ketoglutarate to form glutamate.
   a) Glutamate is then deaminated to give ammonia.
   b) Note that transamination does not provide free ammonia (NH_3).
2. Transamination is important for formation of non-essential amino acids (through reactions of α-ketoacids with glutamate).

D. Role of pyridoxal phosphate in transamination:

Pyridoxal phosphate (PLP) is a coenzyme that acts as an intermediate carrier of amino group. The figure shows the inter-conversion of pyridoxal phosphate and pyridoxamine phosphate during transamination reactions.



Role of pyridoxal phosphate in transamination. Oraby

E. Diagnostic importance of transaminases (ALT and AST):

1. Transaminases are normally intracellular enzymes.
2. The presence of elevated levels of transaminases in the blood indicates damage to cells producing these enzymes. This occurs in certain diseases:
   a) Elevated levels of both **ALT and AST** indicate possible damage of liver cells with subsequent escape of hepatic enzymes into blood
   b) An elevated level of **AST only** suggests damage to heart muscle (myocardial infarction), skeletal muscle or kidney.

II. *Deamination:*

A. Definition:

It is the removal of amino group from amino acids in the form of ammonia (NH_3).

B. **Site:** Mostly liver and kidney.

C. **Types:**

1- OXIDATIVE DEAMINATION:
- By L-glutamate dehydrogenase enzyme.
- By L-amino acid oxidase enzyme.
- By D-amino acid oxidase enzyme.

2- NON-OXIDATIVE DEAMINATION:
- For hydroxy amino acids.

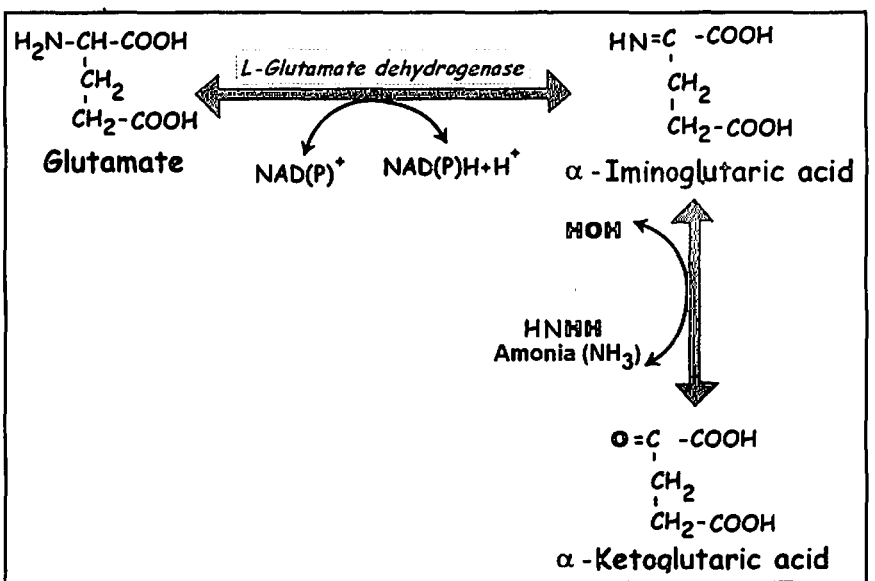
3- HYDROLYTIC DEAMINATION:
- For glutamine and asparagine.
- For adenosine monophosphate (AMP).

1. Oxidative deamination

By oxidative deamination reaction both oxidation (removal of hydrogen) and deamination (removal of ammonia, NH_3) occur altogether. Several enzymes are involved:

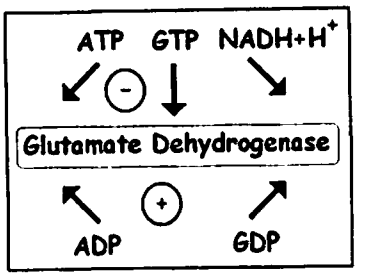
a) **Oxidative deamination by L-glutamate dehydrogenase:**

1) It is highly active enzyme catalyses the deamination of L-glutamate amino acid.
2) This enzyme is present in both cytosol and mitochondria of most tissues.
3) Its coenzyme is either NAD^+ or $NADP^+$.



L-Glutamate dehydrogenase. *Ruby*

4) **Regulation of L-glutamate dehydrogenase:**



i- ATP, GTP and $NADH+H^+$ allosterically inhibit L-glutamate ehydrogenase.

ii- ADP, GDP stimulate it .

iii- Thus, its activity is increased when energy is required

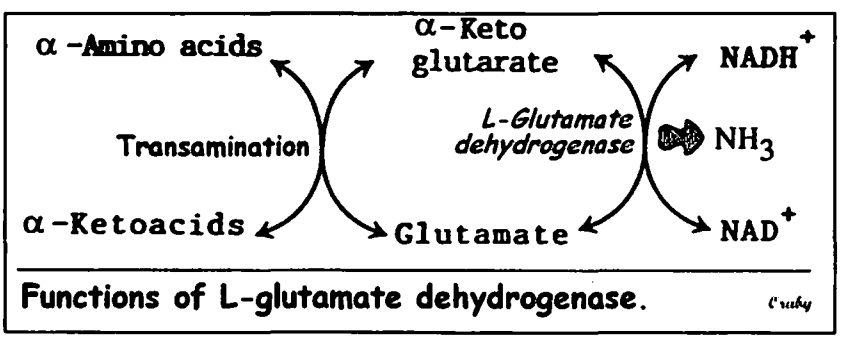
5) **Functions of L-glutamate dehydrogenase:**

i- Removal of NH_2 group of most amino acids as ammonia (NH_3):

- As described above in transamination, the amino group of most amino acids is finally transferred to α-ketoglutarate to form L-glutamate.
- The very active L-glutamate dehydrogenases enzyme then deaminates L-glutamate, producing free ammonia (NH_3).
- Both reactions are called **transdeamination**. This provides a pathway by which the amino group of most amino acids can be released as ammonia.

ii- Formation of non-essential amino acids:

- The reaction catalyzed by L-glutamate dehydrogenase is reversible i.e. α-ketoglutarate can be reaminated by free ammonia to form glutamate.
- Then glutamate can also be transaminated with the desired α-ketoacid to form the corresponding new amino acid.

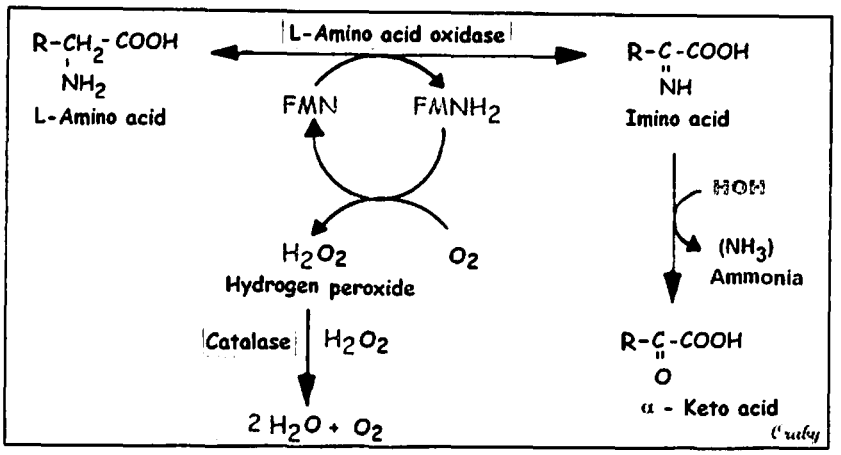


b) **Oxidative deamination by L-amino acid oxidase:**

1) This enzyme is present in a minimal amount in the liver and kidney and it is of little activity.
2) Its coenzyme is FMN.
3) It deaminates most naturally occurring L-amino acids .

3) It deaminates most naturally occurring L-amino acids .

4) H_2O_2 is produced, as a side product .It is highly toxic to the cells. It is hydrolyzed rapidly by catalase into H_2O and O_2.



c) **Oxidative deamination by D-amino acid oxidase:**

1) D-amino acids are found in plants and in cell wall of microorganisms. They are not used in the synthesis of proteins inside the human body.

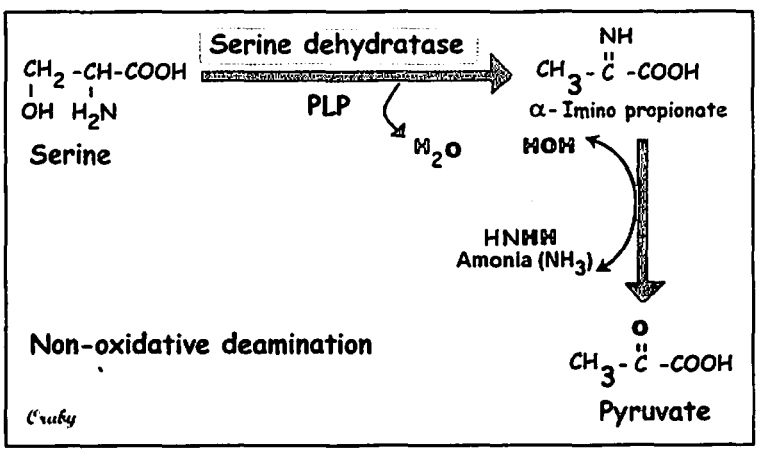
2) Unnatural D-amino acids present in diet are deaminated in the liver by D-amino acid oxidase.

3) Its coenzyme is FAD.

2. Non-oxidative deamination

a) This occurs for hydroxy amino acids e.g. serine, threonine, without removal of hydrogen (non-oxidative)→ Pyruvate.

b) Its coenzyme is pyridoxal phosphate.

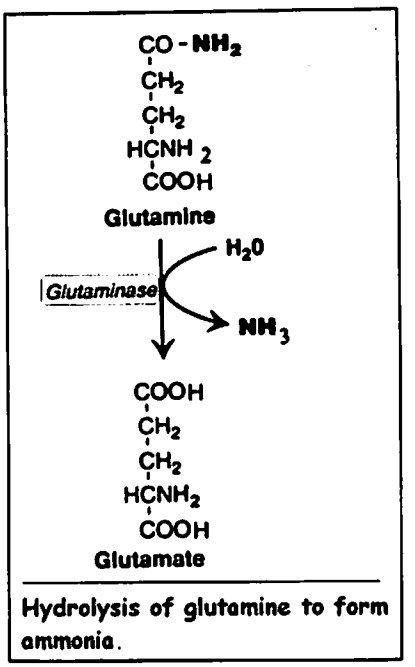


3. Hydrolytic deamination

a) **For glutamine and asparagine:**

1) Both are hydrolytically deaminated by glutaminase and asparaginase respectively.

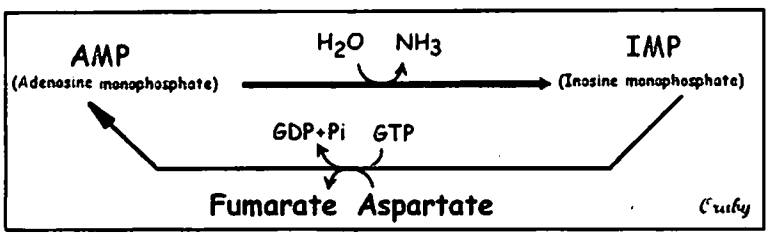
2) Glutaminase enzyme is present in kidney. It produces ammonia (NH_3), which is used in regulation of acid base balance by kidneys.



Hydrolysis of glutamine to form ammonia.

b) **For adenosine monophosphate (AMP) :**

1) In muscles, AMP is hydrolytically deaminated into inosine monophosphate (IMP), producing ammonia (NH_3). This occurs by a series of reactions called: IMP - AMP cycle.



III. *Transdeamination:*

A. **Definition:** It is transamination of most amino acids with α-Keto- glutarate to form glutamate.

B. Then glutamate is deaminated to give ammonia (NH_3).

C. It is the main pathway by which amino group (NH_2) of most amino acids is released in the form of ammonia (NH_3). (Discussed before in deamination by L-glutamate dehydrogenase).

IV. *Decarboxylation:*

A. Decarboxylation (removal of CO_2) of amino acids produces the corresponding **amines**.

B. Some amines have important biologic functions: e.g.

1. Histamine (from histidine) is vasodilator.
2. γ-Amino butyric acid (from glutamate) is neurotransmitter.

C. The resulting amines are further oxidized -after carrying out their functions- by amine oxidase enzymes.

D. Amine oxidase enzymes are two types: monoamine oxidase and diamine oxidase. Both contain pyridoxal phosphate and copper as prosthetic groups.

Ammonia (NH_3)

I. Introduction:

A. Ammonia is a toxic substance especially to the central nervous system.

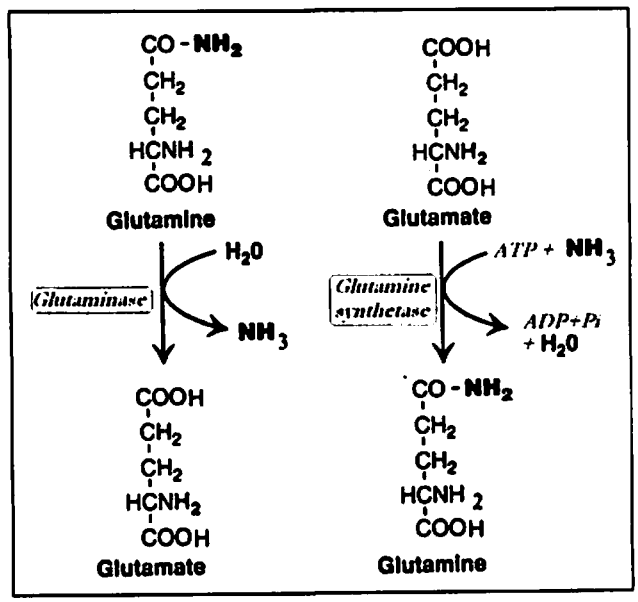
B. Any ammonia formed in the peripheral tissue must be moved to the liver to be converted into urea. This maintains ammonia at low level in circulating blood.

C. **Blood ammonia:** Blood contains traces of ammonia: **10 - 80 ug/dl.**

II. Sources and fate of ammonia:

A. Sources:

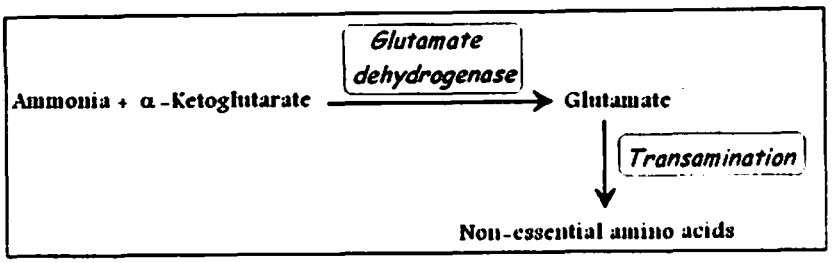
1. **Transdeamination of amino acids:** Many tissues, particularly **liver**, form ammonia from amino acids by transdeamination.
2. **Glutamine:** The **kidneys** form ammonia from glutamine by glutaminase enzyme. Most of this ammonia is used in regulation of acid base balance by the kidneys.



3. **Purines and pyrimidines** metabolism.
4. **Various nitrogenous compounds e.g.** monoamines that act as neurotransmitters.
5. **In intestine,** ammonia is produced by the action of intestinal bacterial enzymes on:
   a) Dietary amino acids.
   b) Urea secreted into the intestine.

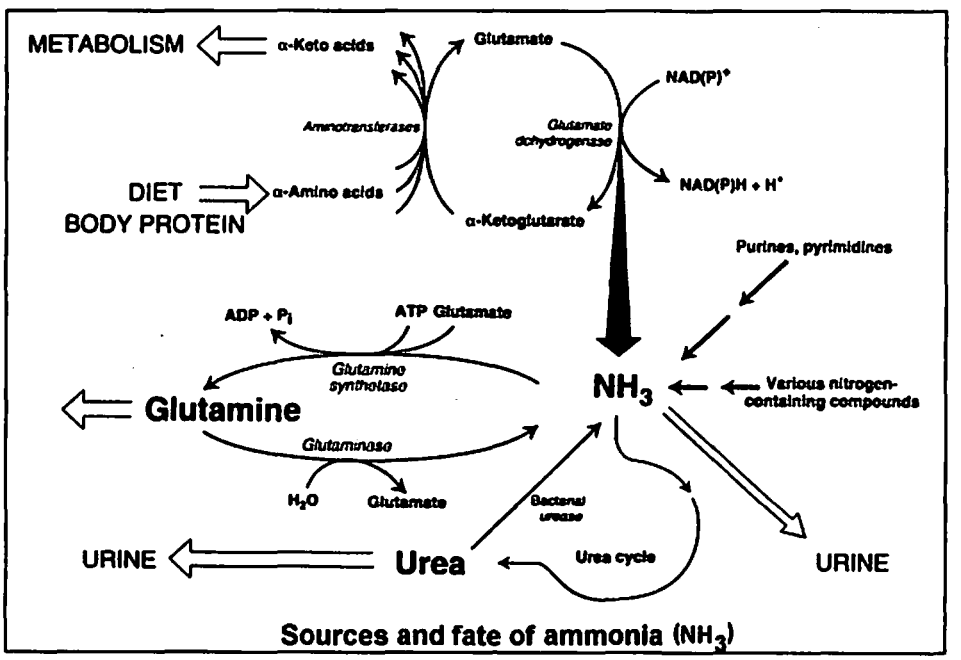
B. Fate of ammonia:

1. **Formation of non-essential amino acids:** Through trans-deamination reactions.



2. **Formation of urea:** It is the main pathway by which the body can get rid of ammonia.
3. **Excretion in urine**
4. **Formation of glutamine:**
   a) Glutamine synthetase is a mitochondrial enzyme present in many tissues as kidney and brain.
   b) Glutamine has the following functions:
   1) **Regulation of acid base balance: In kidney,** glutamine is deaminated by glutaminase enzyme, releasing ammonia again. Ammonia is used by the kidneys in regulation of acid base balance.
   2) **Removes the toxic effect of ammonia In brain:** Ammonia + Glutamate → Glutamine. See ammonia intoxication.
   3) Glutamine is the **source of N_3 and N_9** of purine bases.
   4) Glutamine is used in **detoxication** of phenyl acetic acid (a toxic substance).

III. ***Ammonia intoxication:*** Will be discussed after the subject of urea.



Urea

I. *Introduction:*

A. Urea (H_2N-CO-NH_2) is the main end product of protein (amino acids) metabolism.

B. Urea formation is the pathway through which the **liver** can convert toxic ammonia into non-toxic urea.

C. **Site of urea formation:**

1. **Liver is the only site for urea formation.**
2. **Then urea is transported by the blood to the kidney to be excreted in urine (urine urea is 20-40 g/day).**

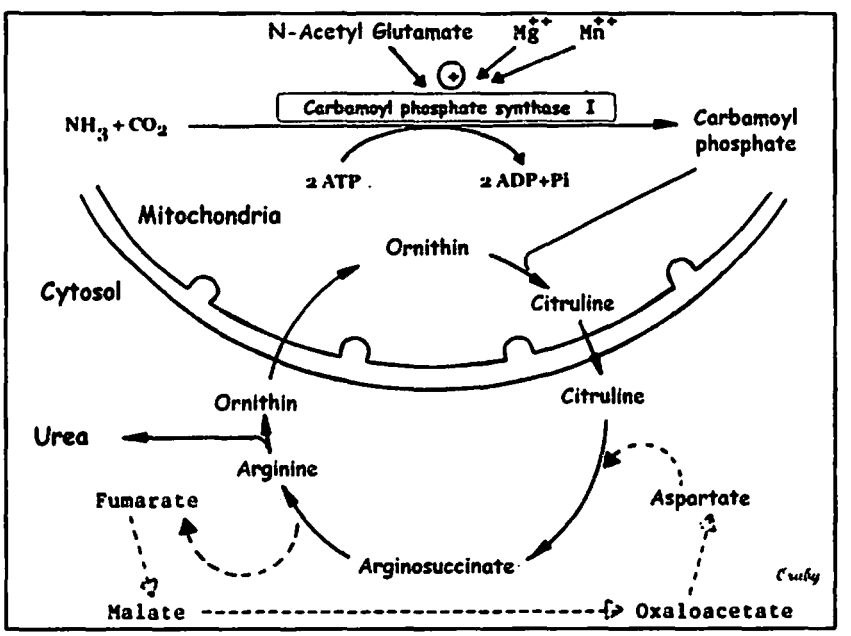
D. **Plasma urea:**

1. **Plasma urea:** is 20-50 mg/dl.
2. **Diagnostic importance of plasma urea determination:**
   a) Measurement of plasma urea is one of the kidney function tests.
   b) In kidney diseases as in renal failure, kidney fails to excrete urea ➔ High blood urea concentration (uremia).

II. *Urea formation:* It is also called Krebs` Henseleit cycle.

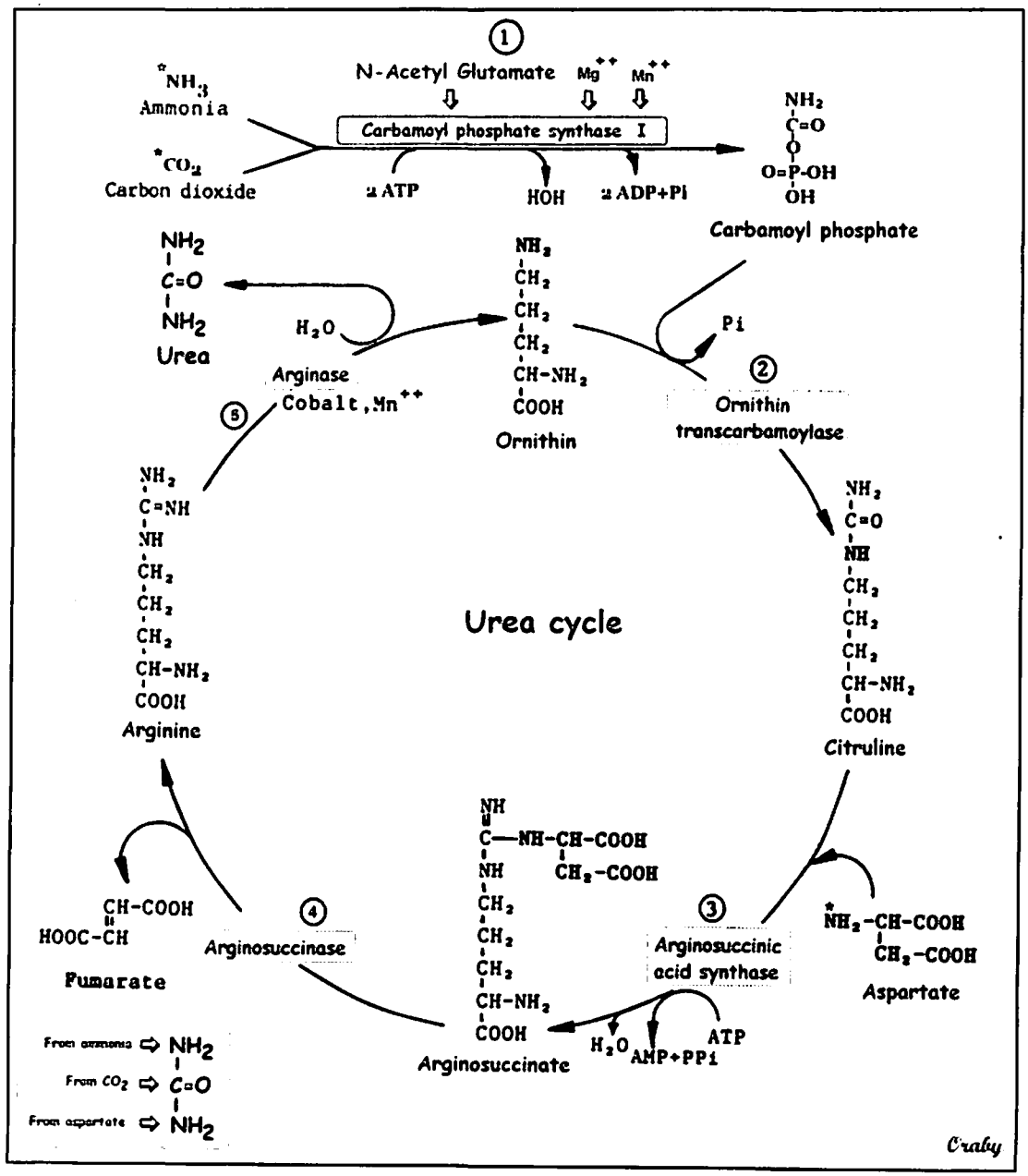
A. **Site: Liver.**

1. The first two reactions occur in **mitochondria** where other reactions occur in **cytosol.**
2. **Six amino acids share in urea cycle:** ornithine, citrulline, arginosuccinate, aspartate and arginine. The 6th one is N-acetylglutamate that acts as allosteric activator of carbamoyl phosphate synthase I.



B. **Steps:**

1. **Formation of carbamoyl phosphate:**
   a) This reaction occurs in mitochondria.
   b) It needs CO_2 (a product of citric acid cycle), ammonia (a product of deamination of glutamate) and phosphate (from ATP).
   c) This reaction is catalyzed by carbamoyl phosphate synthase I. It needs magnesium (Mg^{++}) ions, manganese (Mn^{++}) and N-acetylglutamate as activators.
   d) 2 ATP molecules are used in this reaction, one to provide phosphate and the other to supply energy.



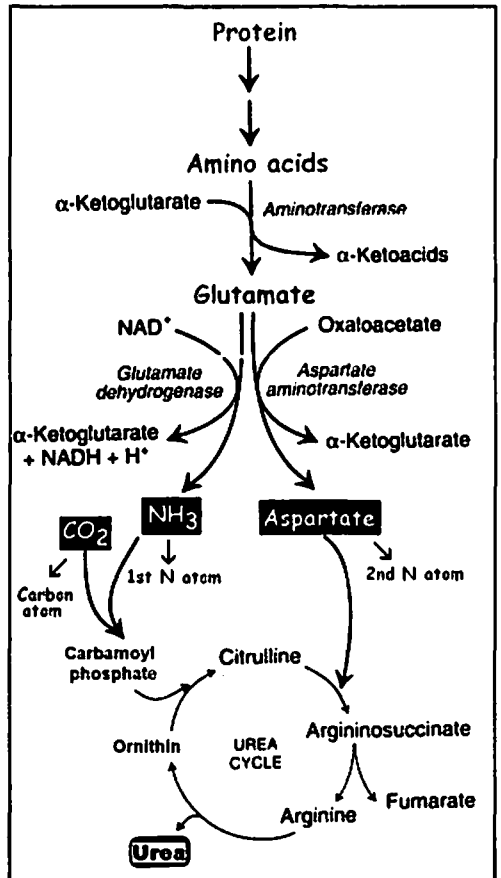
N.B. Carbamoyl phosphate synthase II is a cytosolic enzyme used for pyrimidine synthesis. (see nucleotides metabolism).

2. **Formation of citrulline:**
   a) This reaction also occurs in mitochondria.
   b) Carbamoyl phosphate reacts with ornithine, in the presence of ornithine transcarbamoylase enzyme producing citrulline.
   c) Citrulline then passes to cytosol.
   d) Ornithine is regenerated with each turn of urea cycle.
3. **Formation of arginosuccinate:**
   a) Citrulline reacts with aspartate in the cytosol to form arginosuccinate.
4. **Cleavage of arginosuccinate:**
   a) It is cleaved into arginine and fumarate.
5. **Cleavage of arginine into ornithine and urea:**
   a) Ornithine then passes to the mitochondria to start a new cycle.
   b) Urea passes to the blood to be excreted -by the kidney- in urine.

C. **Three ATP** molecules and four high-energy phosphate bonds are utilized in the reactions.

D. **Sources of different atoms of urea (H_2N-CO-NH_2):**

1. **Carbon atom**: from CO_2.
2. **1st Nitrogen atom**: from ammonia.
3. **2nd Nitrogen atom**: from aspartate.



Flow of nitrogen from amino acids to urea.
Nitrogen atoms of urea are derived from ammonia and aspartate. Carbon dioxide supply carbon of urea. *Cruky*

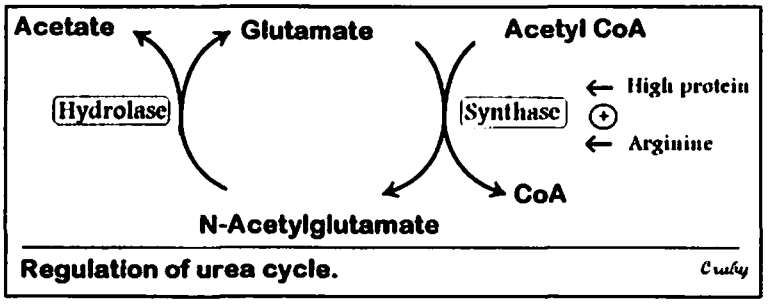
The overall reactions of urea cycle:
Aspartate + NH_3 + CO_2 + 3 ATP ⇨ Fumarate + Urea + 2 ADP + AMP + 2 Pi + PPi + 3 H_2O

E. Fate of urea:

1. Urea diffuses from the liver and is transported by the blood to the kidneys, where it is filtered and excreted in the urine.
2. A portion of the urea synthesized in the liver diffuses from the blood into the intestine and is cleaved into CO_2 and NH_3 by bacterial urease. This ammonia is partly lost in the feces and is partly reabsorbed into the blood.
3. In patients with kidney failure, plasma urea levels are elevated → ↑ transfer of urea from blood to the intestine → ↑ Action of intestinal urease on urea → ↑ ammonia synthesis → **Hyperammonemia**. Oral administration of neomycin antibiotic reduces the number of intestinal bacteria responsible for the NH_3 production.

F. Regulation of urea cycle:

1. The key enzyme of urea cycle is **carbamoyl phosphate synthase I**. It is activated allosterically by N-acetylglutamate, Mg^{++} and Mn^{++} ions.
2. N-acetylglutamate synthesis is stimulated by high protein diet and amino acids especially arginine. This will promote urea cycle.



Regulation of urea cycle. Oraby

G. Relationship between tricarboxylic acid cycle (TCA) and urea cycle:

1. CO_2 needed for urea formation is mostly produced in TCA.
2. Fumarate produced in urea cycle can be oxidized in TCA.
3. Aspartate can give oxaloacetate and vice versa (transamination).
4. ATP needed for urea cycle is derived from TCA

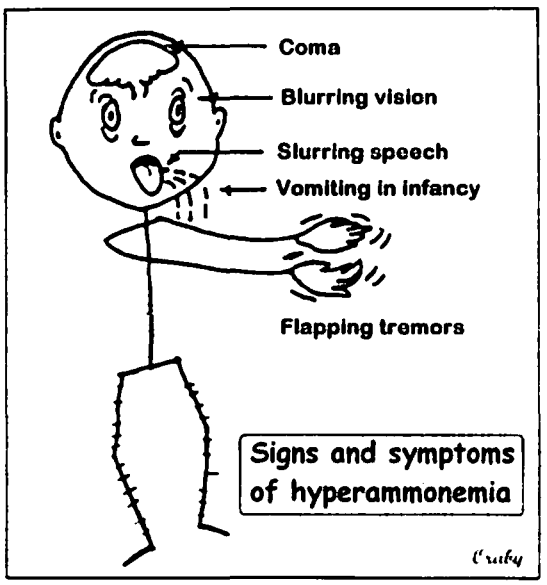
Ammonia intoxication

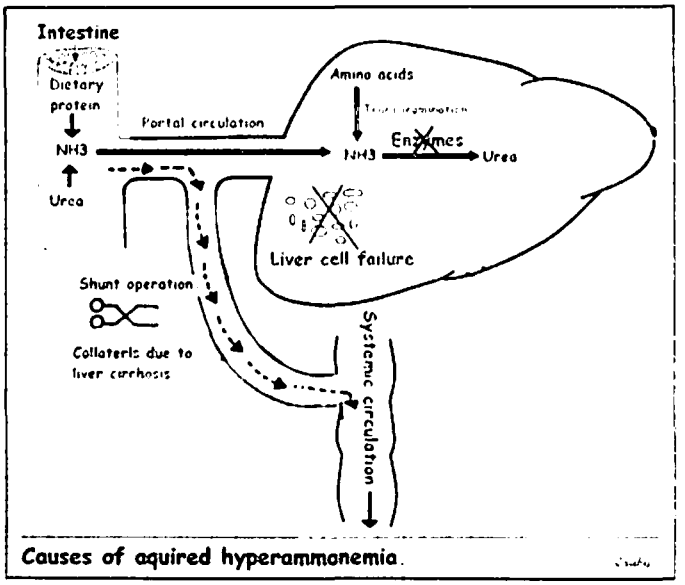
I. Excess ammonia is toxic to the central nervous system. The condition is called ammonia intoxication or hyperammonemia.

II. ***Symptoms:*** Include:

A. Flapping tremors, slurring speech, blurring vision and vomiting in infancy.

B. High concentration of ammonia may cause coma and death.





III. *Types and causes of hyperammonemia:*

A. Acquired hyperammonemia:

1. **Liver cell failure:** The diseased liver cells cannot convert ammonia into urea.
2. **Renal failure.**
3. **Shunt operation** between portal and systemic circulation.
4. **Collaterals** between portal and systemic circulation due to cirrhosis of liver by bilharziasis, hepatitis etc.

B. Inherited hyperammonemia:

Results from genetic deficiency in one of five enzymes of urea cycle → Failure to synthesize urea → Hyperammonemia during the first week after birth ➔ Mental retardation.

1. **The most common inherited enzyme deficiency are:**
   a) **Carbamoyl phosphate synthese I. It results in what is called: Hyperammonemia type I.**
   b) **Ornithine transcarbamoylase. It results in what is called: Hyperammonemia type II.**
2. **Other enzyme deficiencies include:**
   a) **Citrullinemia: Due to deficiency of arginosuccinic acid synthase.**
   b) **Argininosuccinate aciduria: Due to deficiency of argininosuucinase.**
   c) **Argininemia: Due to deficiency of arginase.**

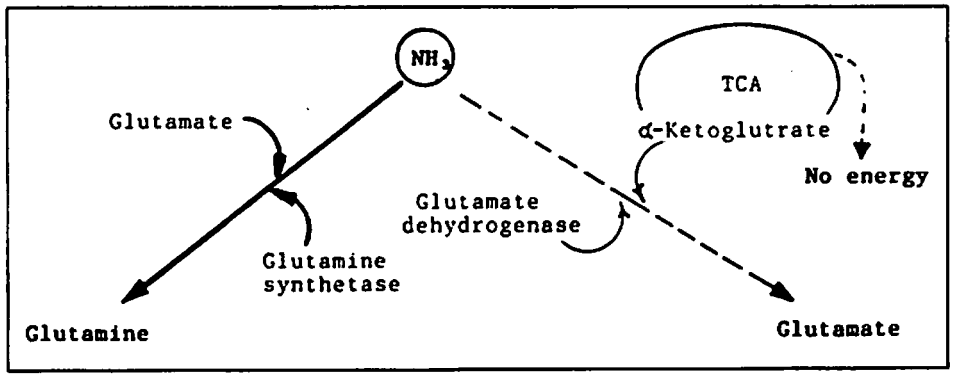
IV. *Mechanism of ammonia intoxication:*

A. At normal blood ammonia level , any ammonia reaches the brain is incorporated into glutamine formation by glutamine synthetase enzyme.

$$NH_3 + \text{Glutamate} \xrightarrow{\text{Glutamine synthetase}} \text{Glutamine}$$

B. In cases of hyperammonemia , ammonia reacts not only with glutamate, but also with α-ketoglutarate by glutamate dehydrogenase enzyme. This depletes α-ketoglutarate which is an essential intermediate of citric acid cycle ➔ Decrease in ATP and energy production ➔ symptoms of ammonia intoxication ➔coma.

$$NH_3 + \alpha\text{-Ketoglutarate} \xrightarrow{\text{L-Glutamate dehydrogenase}} \text{Glutamate}$$



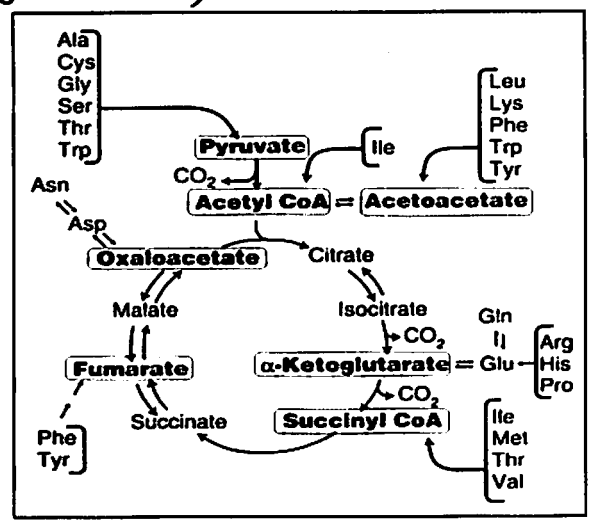
Catabolism of the carbon skeletons of amino acids (Fate of α-keto acids)

I. *Introduction:*

The α-ketoacids (the carbon skeleton) remaining after the removal of the amino group (NH_2) by transamination and deamination of amino acids may undergo:

A. **Reamination** by ammonia (NH_3) to form again the corresponding amino acid (by glutamate dehydrogenase).

B. **Catabolised** to form seven



products: pyruvate, acetyl CoA, acetoacetyl CoA, fumarate, oxalo-acetate, α-ketoglutarate and succinyl CoA.

C. **<u>These products enter different pathways which lead to</u>:**

1. Synthesis of glycogen or **glucose**.
2. Synthesis of **lipids**.
3. Complete **oxidation** into CO_2 and H_2O.

II. *Ketogenic and glycogenic amino acids:*

Amino acids can be classified as ketogenic or glycogenic according to the nature of their metabolic end products.

A. **<u>Ketogenic</u>** amino acids are those whose catabolism gives either acetoacetate , acetoacetyl CoA or acetyl CoA.

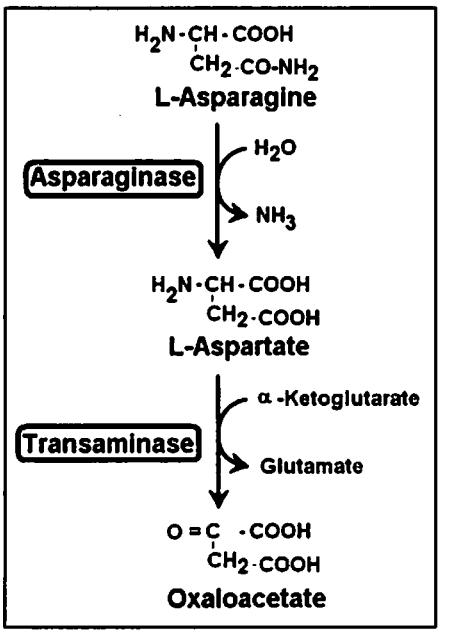
B. **<u>Glycogenic</u>** (or glucogenic) amino acids are those whose catabolism gives pyruvate or one of the intermediate of citric acid cycle.

1. These intermediates are substrates for gluconeogenesis and therefore can give rise to the formation of glycogen or glucose in liver and muscle.
2. 14 Amino acids are glycogenic.

C. **<u>Ketogenic and glycogenic</u>** amino acids are those whose catabolism gives either glycogen or lipid intermediates. 5 Amino acids are glycogenic and ketogenic amino acids.

N.B. Glycogenic amino acids are sometimes named: glucogenic amino acids.

III. *Catabolism of amino acids into α-ketoacids:*



A. **<u>Amino acids forming oxaloacetate:</u>**

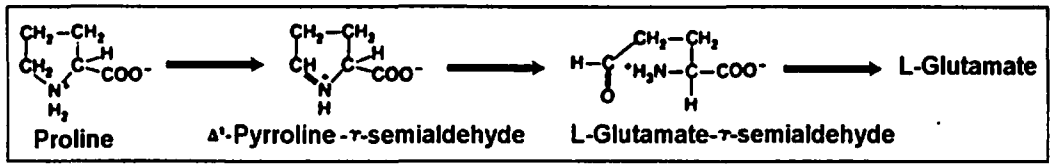
1. These are asparagine and aspartate.
2. **Asparagine** is hydrolysed by the enzyme asparaginase, giving NH_3 and aspartate.
3. **Aspartate** loses its amino group by transamination to form oxaloacetate.

B. **<u>Amino acids forming α-ketoglutarate</u>:**

1. These are glutamine , glutamate, proline, arginine and histidine.

2. **Glutamine** is converted to glutamate and ammonia by the enzymes glutaminase.
3. **Glutamate** is converted to α-ketoglutarate by transamination or through oxidative deamination by glutamate dehydrogenase.

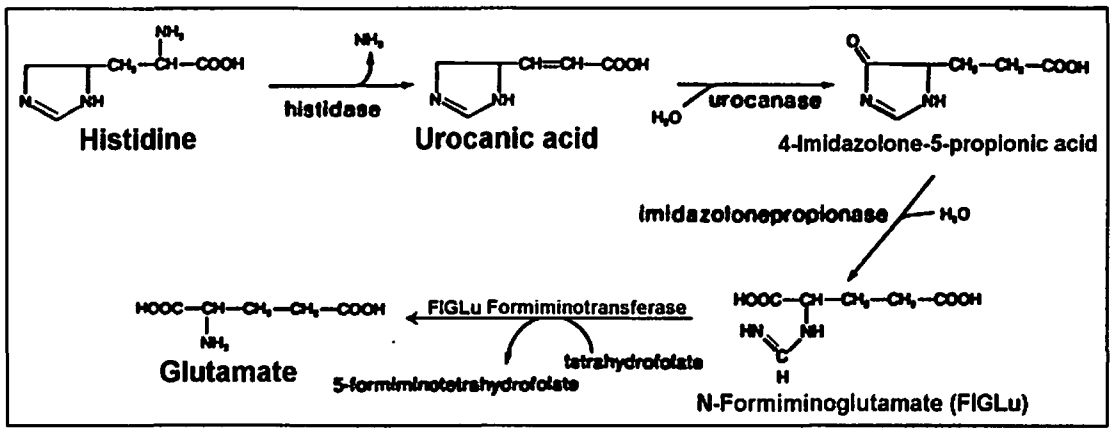
4. **Proline:** is oxidized to Δ 1-pyrroline γ-semialdhyde which is hydrated and oxidized to glutamate . Glutamate is then converted to α-ketoglutarate.



Hyperprolinemia: *a condition of increased blood proline. It is due to defective oxidation of proline to glutamate . It is usually harmless.*

5. **Arginine:** is cleaved by arginase to produce ornithine (see urea cycle page). Ornithine then undergoes transamination to give glutamate-γ-semialdhyde which is converted to α-ketoglutarate as described for proline.

6. **Hisidine :** is deaminated to urocanic acid , which is converted to 4-imidazolone 5-propionate. Hydrolysis of the latter gives **N-formiminoglutamate** (FIGlu) , which donates its formimino group to tetrahydrofolate , leaving glutamate which is then converted to α-ketoglutarate.
   a) **FIGlu excretion test:** Deficiency of folic acid ➔ excretes increased amount of FIGlu in urine. It is **useful test of folic acid deficiency.**

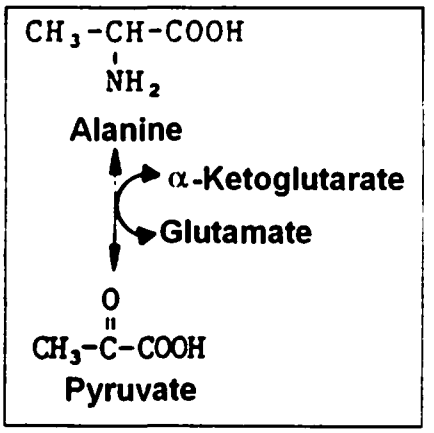


Histidinemia:

* This is a hereditary disease due to deficiency of histidase enzyme.
* It is characterized by mental retardation and speech defects.

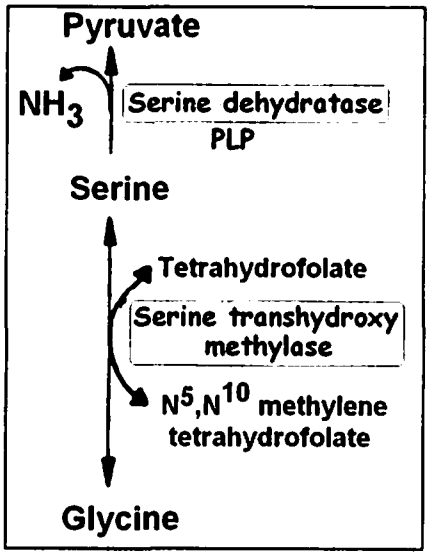
C. **Amino acids forming pyruvate:** These are alanine, serine, glycine, cysteine, cystine, tryptophan and threonine.

1. **Alanine** loses its amino group by transamination to form pyruvate.



2. **Serine** can be converted to:
   a) Pyruvate by the action of serine dehydratase (non-oxidative deamination).
   b) Glycine and N^5, N^{10} methylene tetrahydrofolate.

3. **Glycine** can be converted to serine either by addition of a methylene group from N^5, N^{10} methylene tetrahydrofolate or oxidized to CO_2 and ammonia.



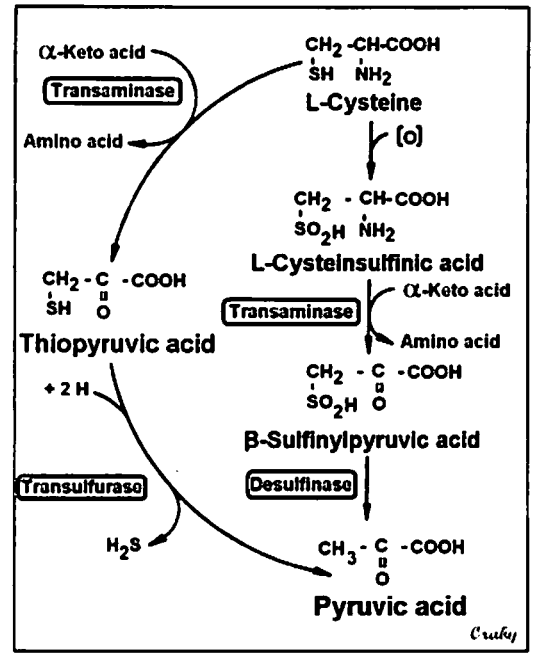
4. **Cystine and cysteine:**
   a) Cystine is reduced to cysteine.
   b) Cysteine then undergoes desulfuration to give pyruvate.

D. **Amino acids forming fumarate:**
These are phenylalanine and tyrosine (see metabolism of phenylalanine and tyrosine).

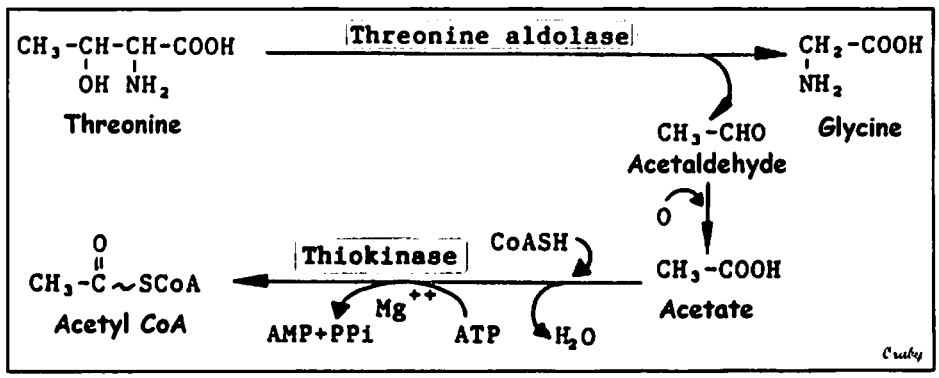
E. **Amino acids forming acetyl CoA and acetoacetyl CoA:**

1. **These are 12 amino acids**
   a) 7 amino acids form pyruvate ➔ acetyl CoA. These are cysteine, cystine, glycine, hydroxyproline, serine and threonine)

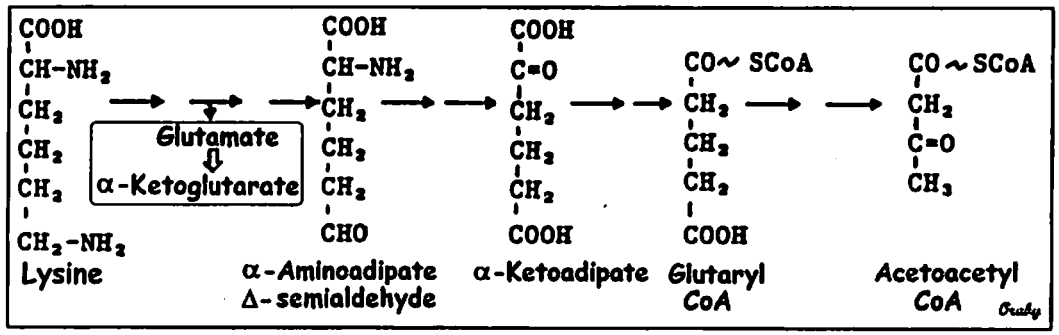
b) 5 amino acids form directly acetoacetate. These are phenylalanine, tyrosine, tryptophan leucine, and lysine).



2. **Catabolism of Phenyl-alanine, tyrosine, tryptophan, leucine and isoleucine:** will be discussed later.
3. **Threonine:**
   a) **Threonine is a ketogenic through its conversion into acetyl CoA:** The major pathway for threonine catabolism in humans is its breakdown to glycine and acetaldehyde. Acetaldehyde is then oxidized into acetate ➔ acetyl CoA, hence, it is ketogenic.

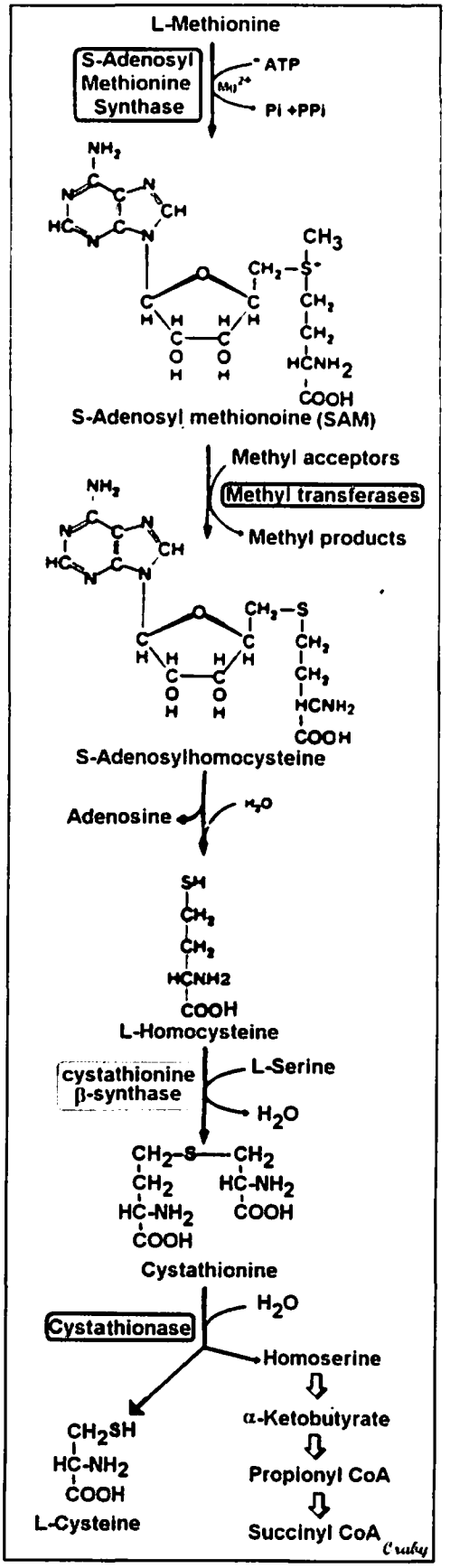


   b) **Threonine is also glycogenic through its conversion to succinyl CoA:** Threonine is dehydrated to α-ketobutyrate ➔ propionyl CoA ➔ to methylmalonyl CoA ➔ succinyl CoA (see oxidation of odd number fatty acids, lipid metabolism part II).
4. **Lysine:** forms α-aminoadipate- Δ-semialdehyde that is finally converted to acetoacetyl CoA.



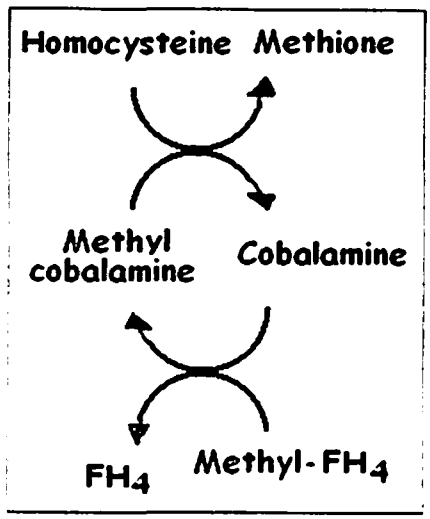
F. **Amino acids forming succinyl CoA:** These are methionine, isoleucine, valine, and threonine.

1. **Methionine:** It is metabolized to **S-adenosyl methionine (SAM),** the major methyl group **donor** in transmethylation reactions.
   a) After giving the methyl group to a methyl acceptor, SAM is converted to S-adenosyl homocysteine, which is then hydrolyzed to homocysteine and adenosine.
   b) Homocysteine has two fates:
      1) Synthesis of cysteine:
         i- Homocysteine can combine with serine forming cystathionine.
         ii- Cystathionine is hydrolyzed to L-homoserine and cysteine.
         iii- Homoserine ➔ α-keto-butyrate ➔ oxidatively decarboxylated to form propionyl CoA ➔ succinyl CoA.
      2) Re-synthesis of methionine:
         i- Homocysteine can accept a methyl group from methyl tetrahydro-folate (Me-H_4-Folate) in a reaction requiring cobalamine (B_{12}) as an intermediate cofactor. This results in synthesis of methionine.
   c) **SAM is a high energy compound** resulting from a condensation of methionine with ATP with hydrolysis of all phosphate bonds in ATP.



1) **Transmethylation** means the transfer of active methyl group from a methyl donor to a methyl acceptor.

2) Examples of methyl acceptors include: nor epinephrine to form epinephrine, guanido acetic acid to form creatine and uracil to form thymine.



Homocystanuria:

This is a hereditary disease due to deficiency of **cystathionine β-synthase enzyme.

**Plasma methionine levels are elevated.*

**There is excessive urinary excretion of homocysteine and s-adenosyl methionine.*

***Signs and symptoms include:** mental retardation, thromboses, osteoporosis and dislocation of eye lenses.*

***Treatment:** feeding a diet low in methionine high in cysteine.*

Cystathioninuria:

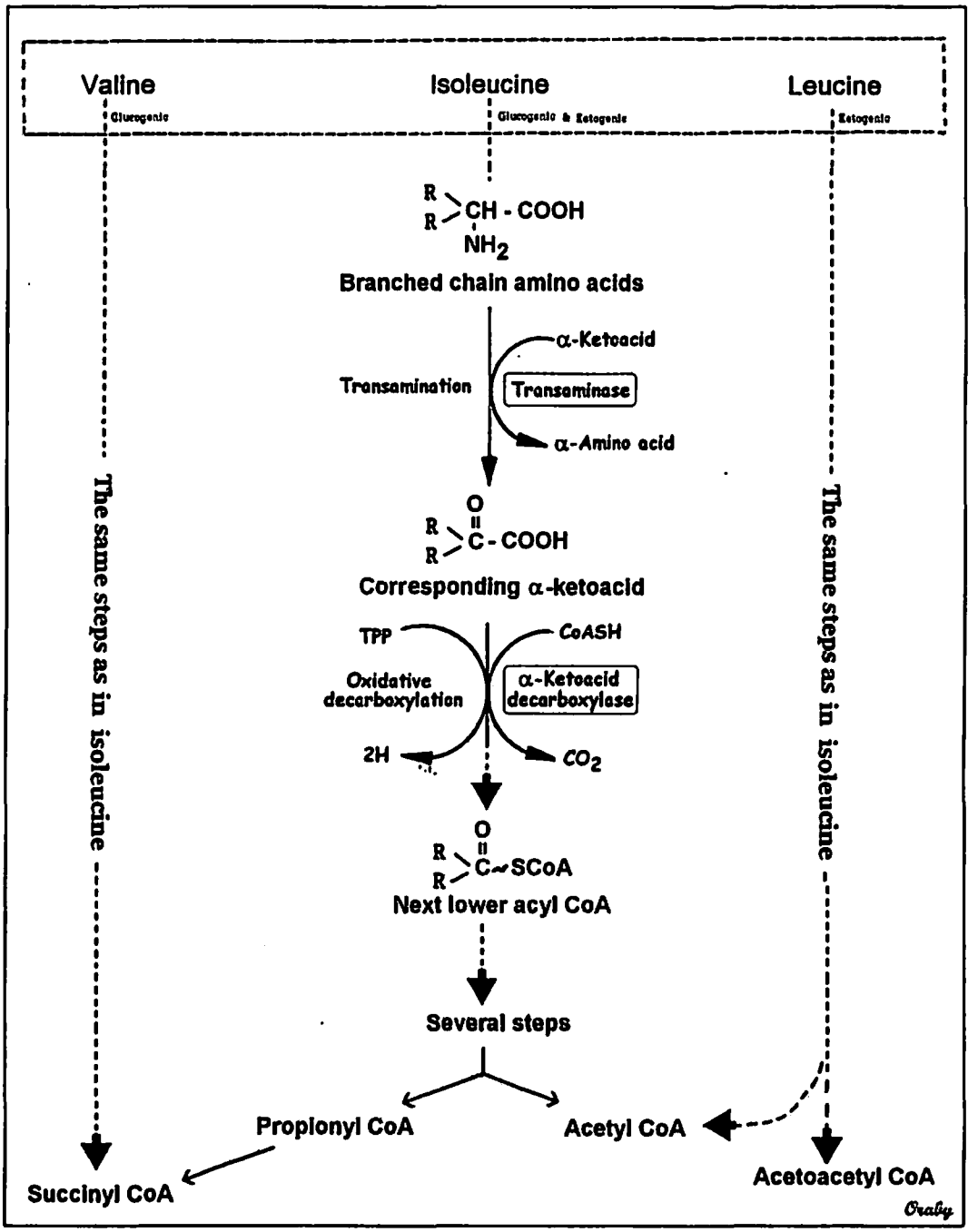
This is an accumulation and excretion of cystathionine due to deficiency of cysta-thioninase enzyme. No clinical symptoms are present.

2. **Isoleucine and valine** will be discussed with catabolism of branched chain amino acids.
3. **Threonine:** has already been discussed.

G. Catabolism of the branched chain amino acids: leucine, isoleucine and valine:

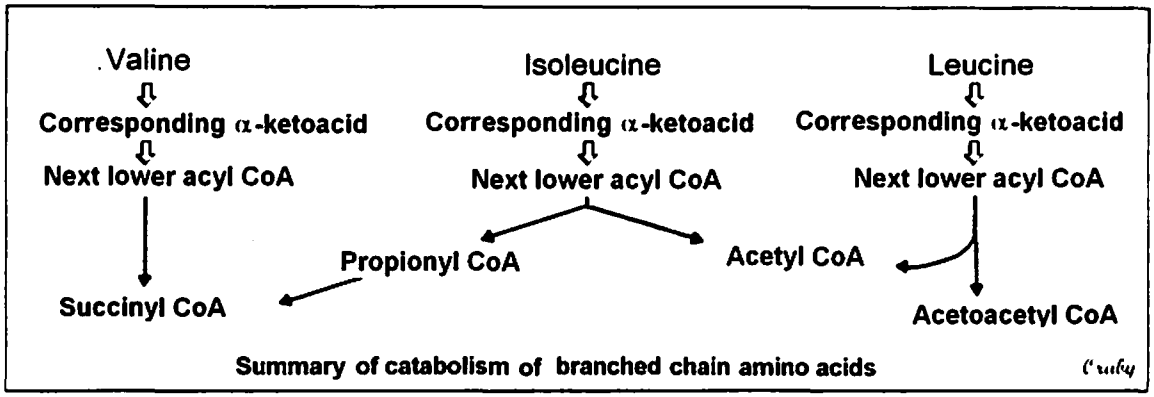
1. All are essential amino acids and their catabolism is discussed as a group.
2. Leucine is pure ketogenic, isoleucine is glycogenic and ketogenic and valine is glucogenic.
3. The transamination of the branched chain amino acids occurs mainly in muscle, brain and adipose tissue. **Liver is deficient** in transaminase required for their transamination.

4. The α-ketoacids, which result from the transamination reactions are oxdatively decarboxylated by a α-ketoacid decarboxylase to give acyl CoA that is less than α-keto acid by one carbon atom. This reaction is similar to that catalyzed by pyruvate dehydrogenase complex.



5. The acyl CoA will be oxidized through several steps to give:
   a) **Succinyl CoA**: this is for valine (glycogenic).
   b) **Acetyl CoA, acetoacetyl CoA**: this is for leucine (ketogenic).

c) **Succinyl CoA, and acetyl CoA**: this is for isoleucine (glycogenic and ketogenic).



Summary of catabolism of branched chain amino acids

Maple syrup urine disease **:**

a) ***Definition:*** *It is accumulation of α-ketoacids of branched chain amino acids and their excretion in urine .*

b) ***Cause:*** *it results from deficiency of oxidative decarboxylation of α-ketoacids.*

c) ***Effects:***

- ***Mental retardation.***
- *Urine has a maple syrup odor or burnt sugar.*
- *Untreated children do not usually live more than 1 year.*

Biosynthesis of nonessential amino acids

I. *Introduction:*

A. All known 20 amino acids are very important to human.

B. They are classified nutritionally into essential amino acids, which cannot be formed in the body and should be taken in diet and non-essential amino acids, which can be synthesized in the body.

II. *Synthesis of alanine, aspartate and glutamate:*

A. They are synthesized from the corresponding α-ketoacids: pyruvate, oxaloacetate and α-ketoglutarate respectively by transamination.

B. Glutamate is unusual in that it can also be synthesized by the reverse of oxidative deamination, **catalyzed by glutamate dehydrogenase.**

III. *Synthesis of glutamine and asparagine:*

A. They are synthesized by amidation in reactions catalyzed by glutamine synthetase and asparagine synthetase.

IV. *Synthesis of proline:*

A. Glutamate is converted into proline by forming glutamate semi-aldehyde. Reduction of the latter gives proline (see proline catabolism)

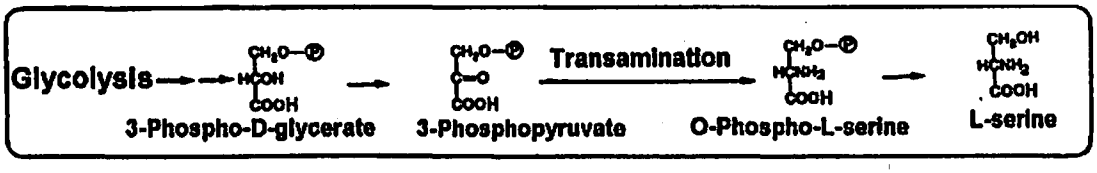
V. *Synthesis of glycine, cysteine and serine:*

A. **Glycine** can be synthesized from serine by removal of a methylene group (see serine catabolism).

B. **Cysteine** is synthesized by 2 successive reactions:

1. Homocysteine combines with serine to form cystathionine.
2. Cystathionine is hydrolyzed to cysteine and homoserine. Homocysteine is derived from methionine (see methionine catabolism)

C. **Serine** is synthesized from 3 Phosphoglycerate (a product of glycolysis) as follows:

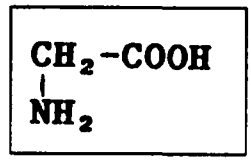


VI. ***Synthesis of tyrosine:*** is synthesized from phenylalanine by the reaction catalyzed by phenylalanine hydroxylase enzyme (see phenylalanine metabolism.

Conversion of amino acids to specialized products

Glycine

I. Glycine is non-essential glycogenic amino acid.

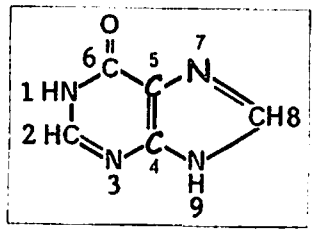


II. ***Functions:*** Glycine is the precursor for:

1- Purine bases
2- Glutathione
3- Creatine
4- Bile salts
5- Heme (hemoglobin)
6- Serine amino acid
7- Collagen
8- Hippuric acid
9- Neurotransmitter
10- Glyoxylic acid

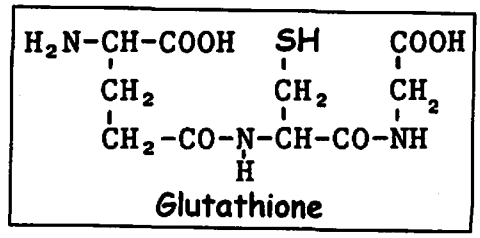
A. **Purine bases:**

Carbon atoms NO .4, 5 and nitrogen atom NO. 7 are derived from glycine.



B. **Glutathione:** It is also called γ-glutamyl, cysteinyl glycine.

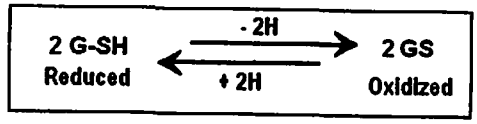
1. **Structure:** Glutathione is a tripeptide formed of three amino acids: glutamate, cysteine and glycine.



2. **Synthesis:** It is synthesized in two steps catalyzed by γ-glutamyl cysteine synthetase and glutathione synthetase (see amino acids absorption page 5).

3. **Forms of glutathione:**

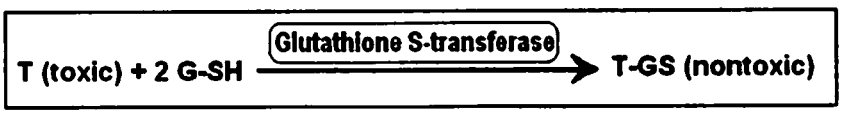
a) Two Forms are present: reduced (G-SH) and oxidized (G-S). The –SH group indicates the sulfhydryl group of the cysteine and it is the most active part of the molecule.



b) G-S is converted to G-SH by the enzyme glutathione reductase and $NADPH^+ + H^+$, a reduced coenzyme produced in the first reaction of pentose phosphate pathway (see carbohydrate oxidation part II).

4. **Functions of glutathione:** It is a reducing substance:

a) G-SH is an important **defense mechanism against certain toxic compounds** (T) as some drugs and carcinogens i.e. substances cause cancer. It combines with them to produce non toxic compounds:



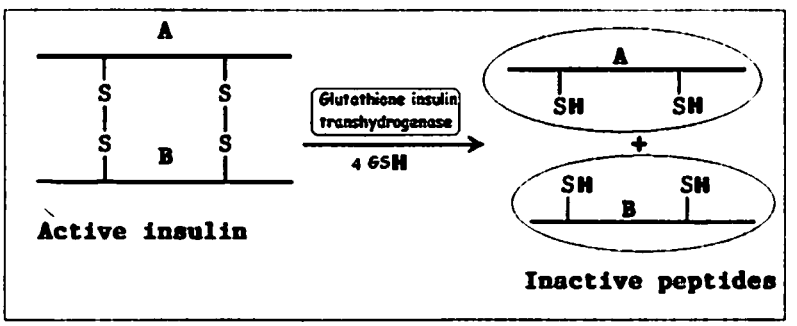
This reaction needs glutathione S-transferase enzyme that is present in high concentration in liver.

b) **G-SH breaks down the toxic hydrogen peroxide** (H_2O_2), which causes damage to cell walls, e.g. protect RBCs from hemolysis (see pentose phosphate pathway, part II).

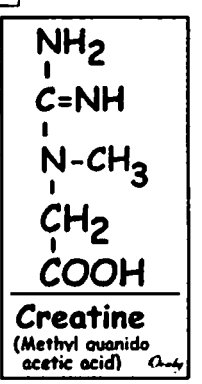
c) G-SH has a role in absorption and **transport of amino acids** across cell membranes (see amino acid absorption).

d) G-SH acts as an **activator for some enzymes.**

e) G-SH has a role in **inactivation of insulin** hormone in reaction needs insulin Glutathione transhydrogenase enzyme. This enzyme is present in liver.



C. **Creatine** (N-methyl-guanidoacetate):

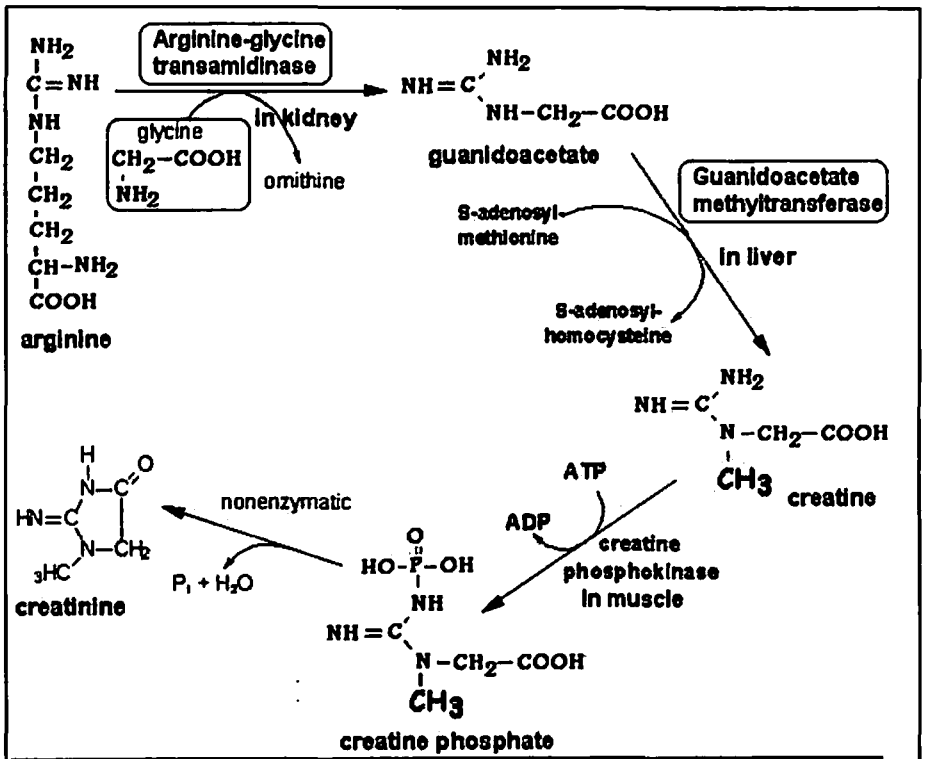


1. **Functions of creatine:**

a) Creatine is phosphorylated to **creatine phosphate** (by creatine kinase enzyme using ATP as a phosphate donor). **This occurs in muscles.**

b) Creatine phosphate acts as a store of high energy phosphate in muscles and used during muscle exercise (as it can give phosphate to ADP to form ATP).

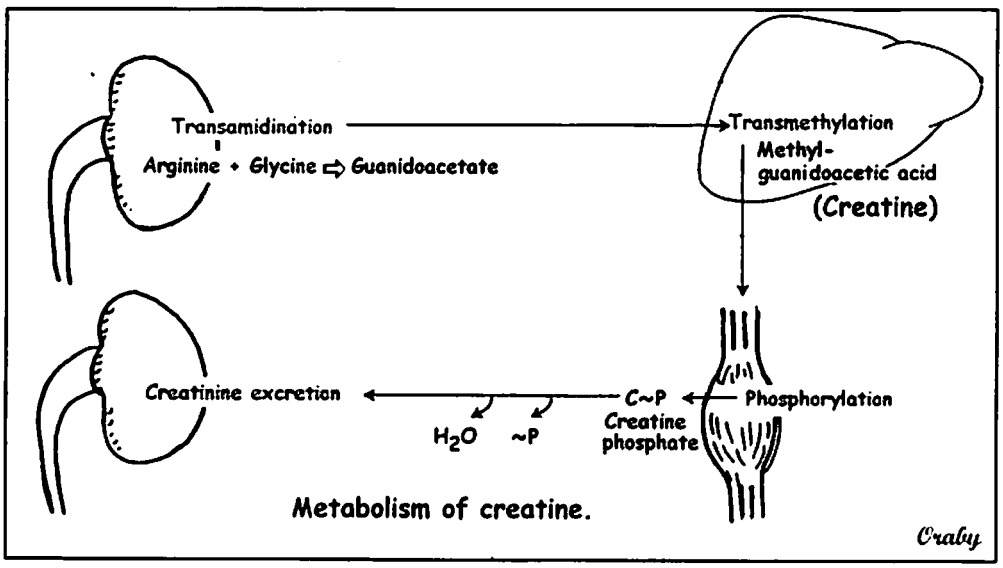
2. **Synthesis of creatine:** It is synthesized from three amino acids: **glycine, arginine, and methionine.** This occurs by two reactions in kidney and liver.
   a) **In kidney:** the first reaction is **transamdination** i.e. transfer of gaunido group, H_2N-[C=NH]-NH_2 from arginine to glycine to form guanidoacetate.
   b) **In liver:** the second reaction is transmethylation (i.e. transfer of methyl group from S-adenosyl-methionine to guanidoacetate to form methyl guanidoacetate [creatine].



Synthesis and metabolism of creatine. *Oraby*

3. **Creatine kinase enzyme (CK):** Also called creatine phospho kinase (CPK):
   a) This enzyme catalyses the formation of creatine phosphate.
   b) It is present in 3 forms (isoenzymes) in serum:
      1) **CK-MM:** derived mainly from skeletal muscles and its serum level is elevated in muscle disease (muscle atrophy).
      2) **CK-MB:** derived mainly from heart muscle and its serum level is elevated in recent myocardial infraction.
      3) **CK-BB:** derived from brain and its serum level is elevated in damage of brain cells.

4. **Degradation of creatine phosphate ➔ (gives creatinine):**
   a) Creatine phosphate loses water and phosphate molecules to form a substance called **creatinine** (= anhydrous creatine).
   b) **Creatinine** is the end product of creatine metabolism and is normally rapidly removed from the blood and excreted by the kidney in urine.
5. **Diagnostic importance of determination of plasma creatinine:**
   a) Estimation of plasma creatinine (0.6 - 1.2 mg/dl) is used as kidney function test.
   b) High blood creatinine (and urea) levels are sensitive indicators of renal impairment e.g. renal failure.



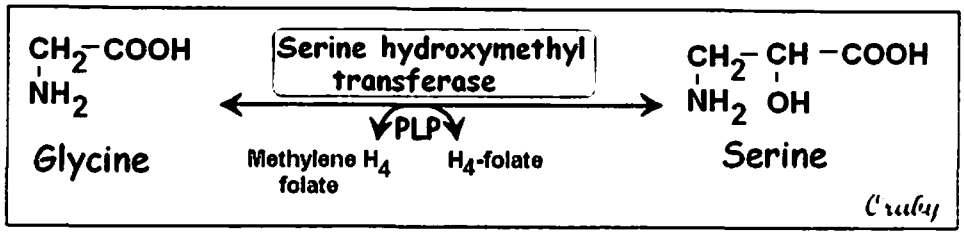
Metabolism of creatine.

D. **Bile salts:**
   1. These are sodium and potassium salts of glycocholic acid.
   2. Glycine is conjugated to cholic acid to form glycocholic acid (see digestion in part I).

E. **Heme:**
   1. Heme is the pigment, which combines with globin protein to form hemoglobin.
   2. **Glycine** reacts with succinyl CoA to form a substance called amino levulonic acid (ALA) a reaction needs pyridoxal phosphate. ALA is finally converted to heme (see hemoglobin metabolism).

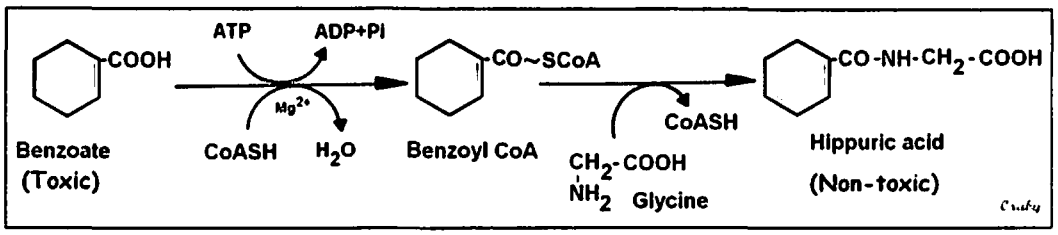
F. **Serine:**

Methylene H_4 folate is used as donor of one carbon fragment ($-CH_2OH$). It gives this carbon to glycine to form serine. The reaction is reversible.



G. Hippuric acid:

Glycine conjugates with the toxic compounds like benzoate (an additive used to preserve foods) to form the non-toxic hippuric acid. This occurs in the liver.

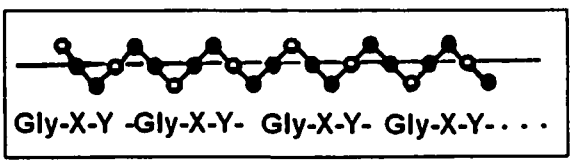


H. Neurotransmitter:

Glycine acts as an inhibitory transmitter in spinal cord and medulla.

I. Collagens:

1. Collagens are the main proteins of connective tissue.

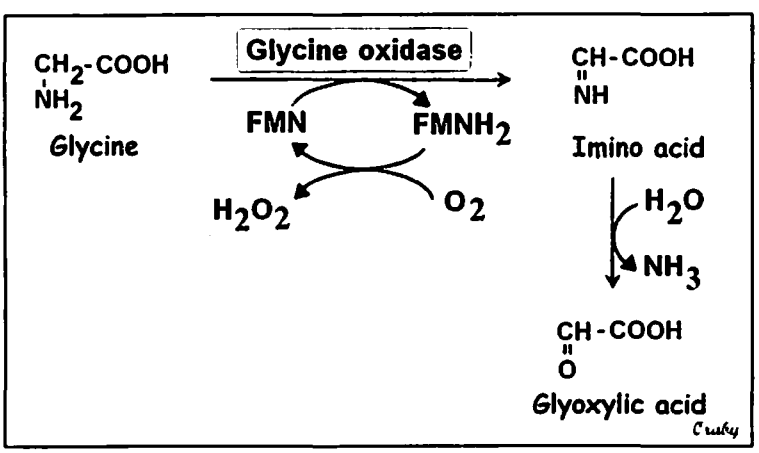


2. All collagen types have a triple helical structure.
3. Each helix is formed of 3 amino acids.
4. Glycine is present at every third position.

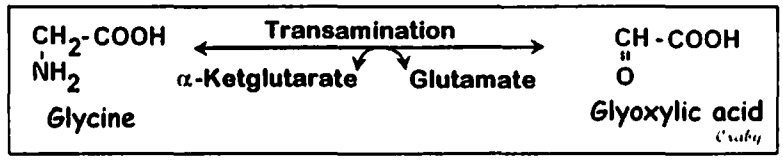
J. Glyoxylic acid

1. **Synthesis:** Glyoxylic acid is formed from glycine by 2 mechanisms: oxidative deamination and transamination:

a) Oxidative deamination:

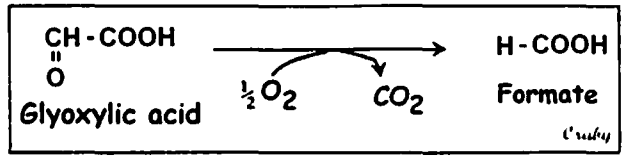


b)Transamination:



2. Fate of glyoxylic acid:

a) Formation of formate: (by oxidative decarboxylation).



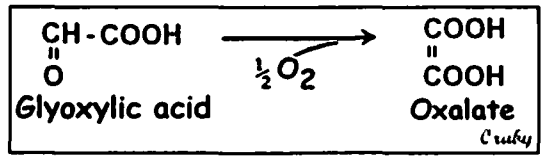
b) Formation of glycine: By reamination (transamination).

Primary hyperoxaluria:

It is a metabolic disease characterized by excessive excretion of oxalate unrelated to dietary intake of oxalate. This leads to formation of urinary oxalate stones.

This condition usually ends by death in early life from either renal failure or hypertension.

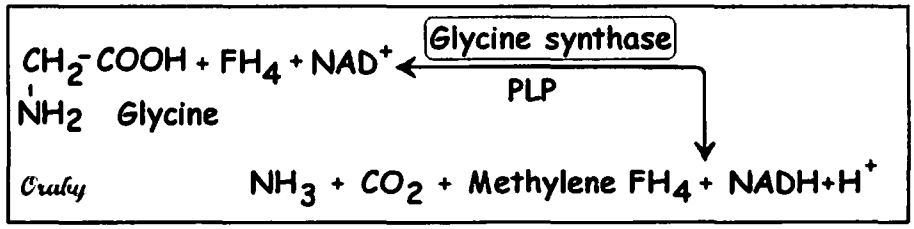
Causes: *failure of conversion of glyoxylic acid into formate or glycine. This leads to oxalate formation.*



Glycinuria

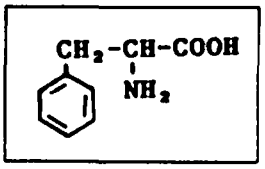
- It is a rare dominant x-linked disease characterized by excess urinary excretion of glycine.
- *It leads to formation of oxalate renal stones.*
- *Glycinuria is due to defect in renal tubular reabsorption of glycine.*

III. ***Catabolism of glycine:*** this is done mainly by the action of the enzyme called: glycine cleavage system (glycine synthase).



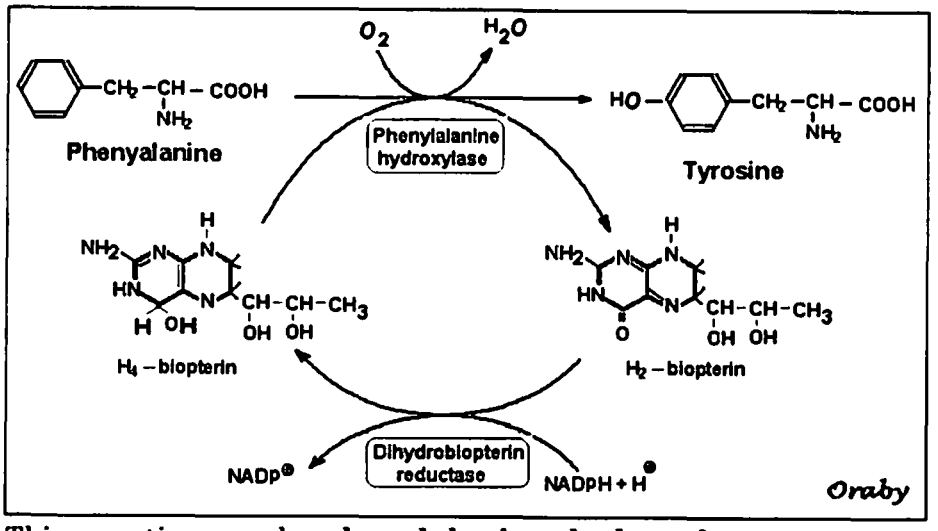
Phenylalanine

I. Phenylalanine is a ketogenic and glycogenic essential amino acid.



II. ***Functions:*** phenylalanine is the precursor for **Tyrosine.**

A. Phenylalanine can be converted to tyrosine **mainly in liver** as follows:



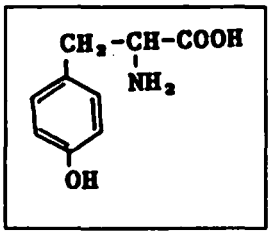
B. This reaction needs **phenylalanine hydroxylase** enzyme and **tetrahydrobiopterin** as coenzyme. This results in the formation of dihydrobiopterin (DHB) which must be regenerated by dihydrobiopterin reductase enzyme with $NADPH^{\cdot} + H^{\cdot}$ as coenzyme.

C. Deficiency of either phenylalanine hydroxylase or dihydrobiopterin reductase results in a disease called: **phenylketonuria.**

Tyrosine

I. Tyrosine is a ketogenic and glycogenic non- essential amino acid.

II. Tyrosine becomes essential in case of deficiency of phenylalanine hydroxylase enzyme.



III. ***Functions:*** Tyrosine is the precursor for:

1-Catecholamines.

2-Melanin pigments.

3-Thyroid hormones.

4-Phenol, cresol and tyramine (decarboxylation products): these are putrefactive substances that produced by the action of bacteria present in large intestine on tyrosine.

A. **Catecholamines:** These are dopamine, norepinephrine and epinephrine.

1. **Functions of catecholamines:**

a) **Neurotransmitters:** Norepinephrine and dopamine act as neurotransmitters in the brain and at most sympathetic postganglionic endings.

b) **Regulation of metabolism:** e.g.

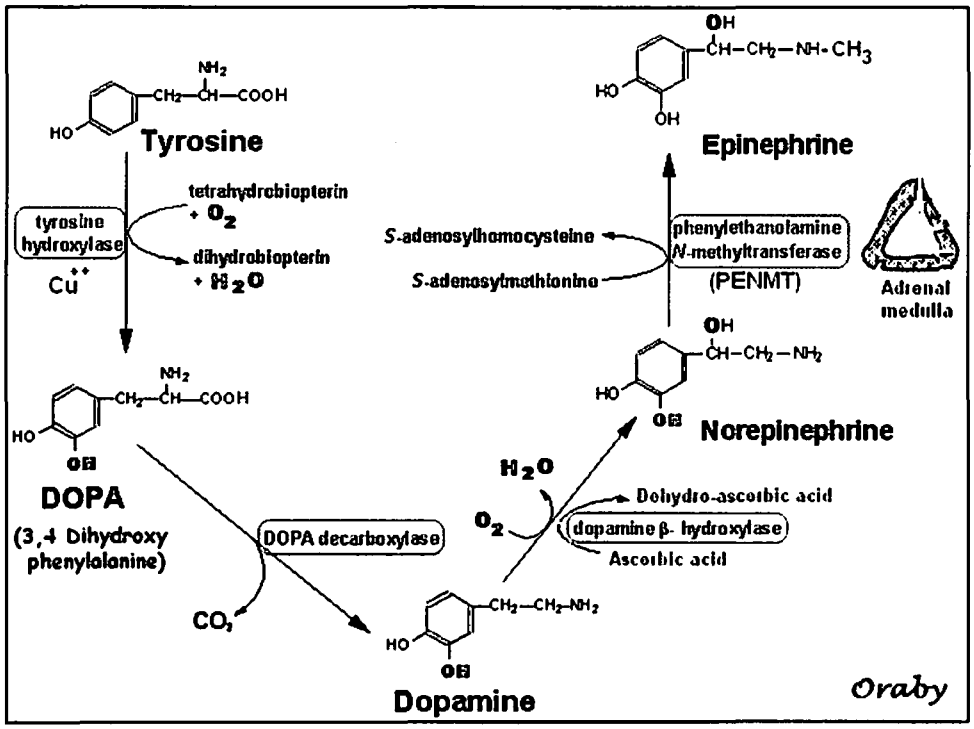
1) Breakdown of glycogen (glycogenolysis) and lipids (lipolysis).

2) Increase of output of the heart and blood pressure.

3) Relaxation of smooth muscles of bronchi and intestine.

2. **Synthesis of catecholamines:**

They are synthesized from tyrosine at storage sites: adrenergic neurons and chromaffrin cells of adrenal medulla.



a) **In neurons:** norepinephrine and dopamine are synthesized from tyrosine as follows:

1) Tyrosine is first hydroxylated to form 3,4 dihydrophenylalanine (**DOPA**) in a reaction similar to that of hydroxylation of phenylalanine.

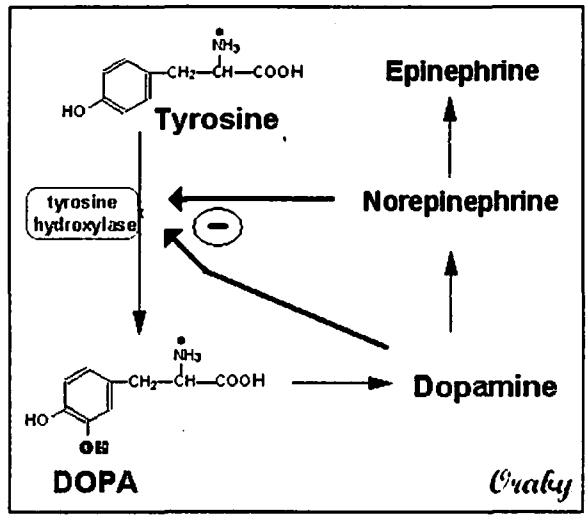
2) **DOPA** is then decarboxylated to dopamine, which is hydroxylated to norepinephrine by dopamine B-hydroxylase, an enzyme containing copper.

b) In adrenal medulla:

1) synthesis of catecholamines is similar to synthesis in neurons.
2) In addition adrenal medullary cells, contain phenylethanolamine N-methyltransferase (PNMT). This enzyme catalyzes the conversion of norepinephrine to epinephrine [by transmethylation].

3. Regulation of synthesis:

Tyrosine hydroxylase is the key enzyme. It is inhibited by feed back inhibition by either dopamine or nor epinephrine.



4. Catabolism of catecholamines:

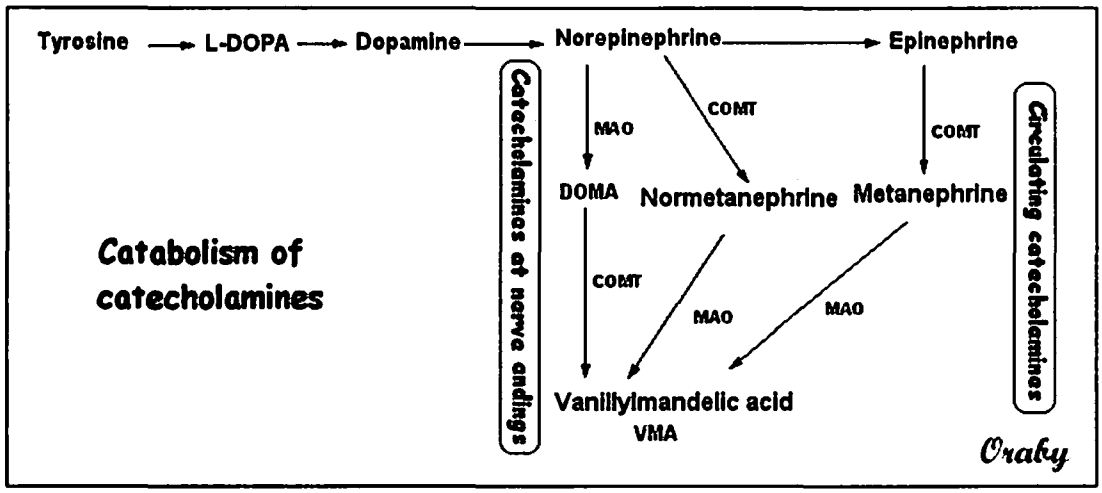
a) Enzymes that inactivate catecholaminse: are two enzymes;

1) **Monoamine oxidase (MAO):** It is present mainly in the mitochondria of adrenergic nerve endings.
2) **Catechol-ortho- methyl transferase (COMT):** which is present in all tissues, with high concentration in the liver and kidney , but not found in the nerve endings.

b) Mechanism of catecholamines catabolism:

1) **At nerve endings:** nor epinephrine is oxidatively deaminated by monoamine oxidase **(MAO)** to 3,4 dihydroxymandelic acid **(DOMA)**, which enters the circulation and converted to VMA.
2) **Circulating epinephrine and norepinephrine:** Are methylated by **COMT** to metanephrine and normetanephrine. These metabolites undergo:
   - They are mostly excreted as such in urine. They can be used as index of the rate of catecholamines secretion.

- MAO may oxidize metanephrine and normetanephrine to **vanillylmandelic acid (VMA)**, which are then excreted in Urine.
- They may conjugate with sulphate and glucuronic acids, and then excreted in urine (smallest amount).

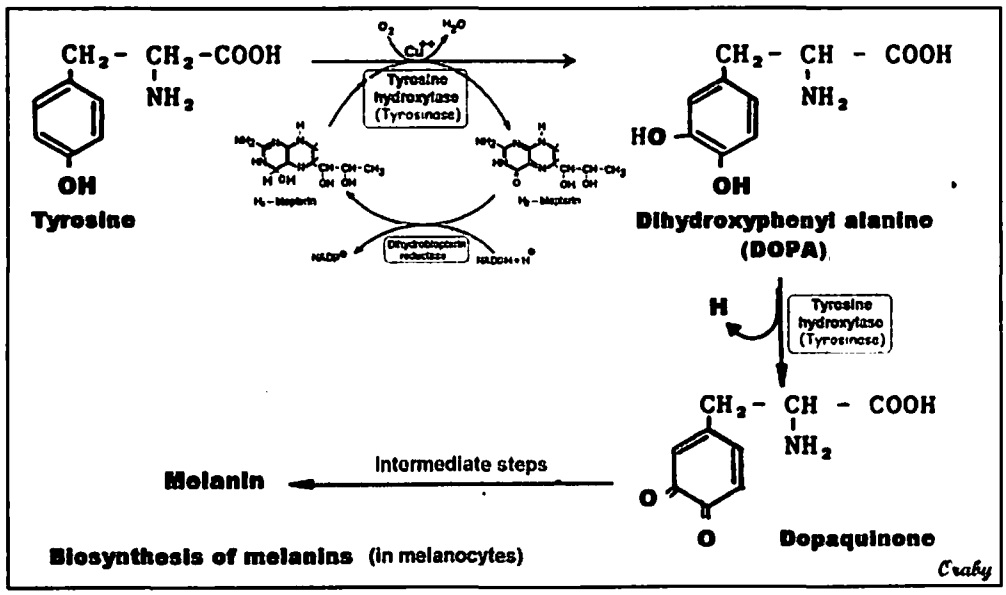


- *Measurement of **urinary VMA** is used as indicator in the diagnosis of adrenal tumors **(pheochromocytomas)** that produce huge amounts of catecholaminse.*

B. Melanin pigments:

1. Synthesis of melanins:

In the skin, melanins are synthesized in melanocytes (pigment forming cells) by tyrosine hydroxylase (tyrosinase) enzyme.



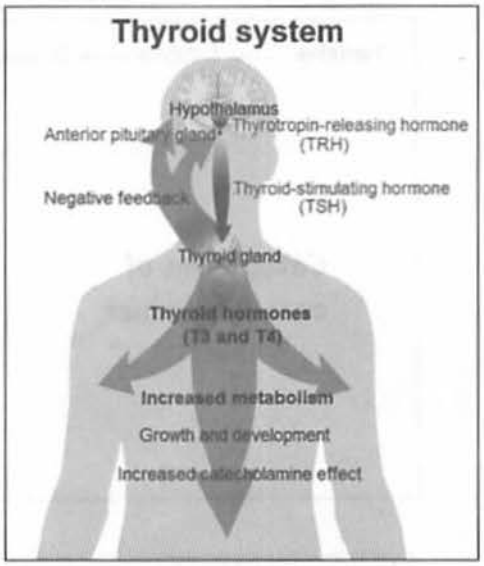
Functions of melanin:

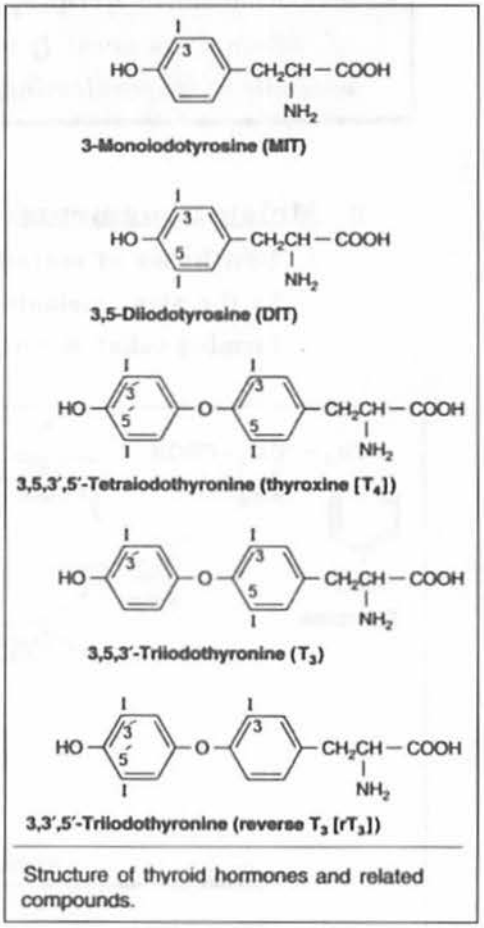
a) Melanins are pigments present in many tissues particularly in the eye (iris), hair and skin.

b) Melanins are synthesized to protect underlying cells from the harmful effects of sunlight.

C. Thyroid hormones:

1. **Functions of thyroid hormones:** The two major hormones produced by thyroid gland are **thyroxin** (T_4) and triiodothyronine (T_3).
   a) They increase **heat production and oxygen consumption** in most tissues through stimulation of ATPase activity.
   b) They regulate the **growth of long bones** (together with growth hormone).
   c) They affect **protein synthesis** through stimulation of DNA in the nucleus of cells.
   d) They increase catecholamine effect.
2. **Synthesis of thyroid hormones:**
   a) Synthesis and release of T_3 and T_4 are stimulated by **thyroid stimulating hormone** (TSH), released from the pituitary gland.
   b) TSH secretion is under control of **thyrotropin releasing hormone** (TRH) which is a tripeptide hormone produced in the hypothalamus.
   c) By feedback mechanism, increased levels of plasma T_3 and T_4 inhibit the secretion of TRH from hypothalamus and TSH from pituitary gland.

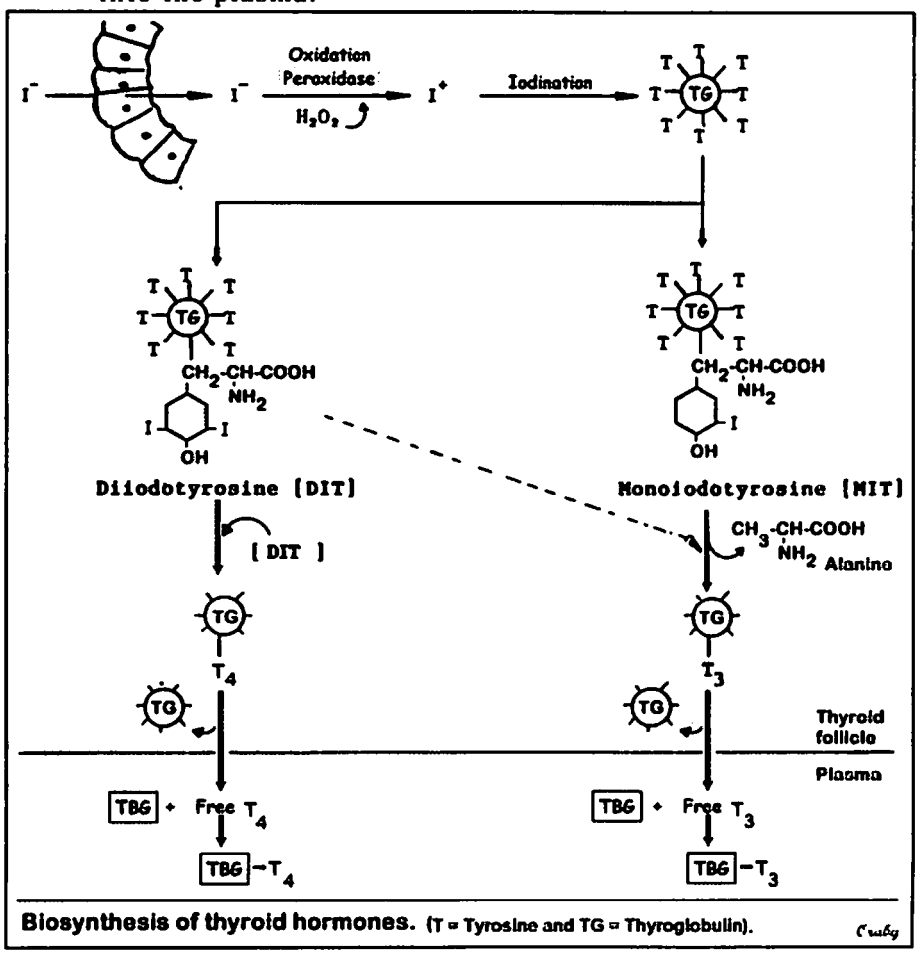




Structure of thyroid hormones and related compounds.

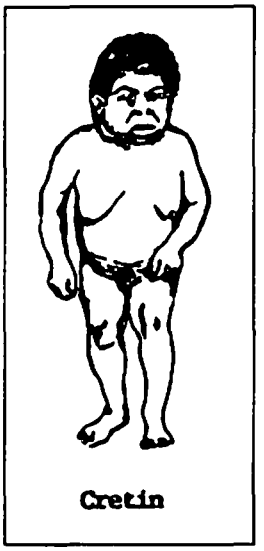
e) ***Steps* of thyroid hormones *synthesis:***

1) The thyroid gland contains many follicles, each composed of a shell of single layered cells surrounding a central space filled with glycoprotein called: thyroglobulin (TG).
2) Thyroglobulin contains about 150 tyrosine residues.
3) Iodide ions (I^-) can be taken up by thyroid cells and oxidized into higher value state (positive ions, I^+). This needs H_2O_2 and thyroid peroxidase enzyme.
4) I^+ is then incorporated into the tyrosine residues of TG.
5) The resulting monoiodotyrosine and diiodotyrosine residues react together to give one of the following compounds:
   a. Tetraiodothyronine [thyroxin, T_4].
   b. Triiodothyronine [T_3].
   c. Reverse triiodothyronine [rT_3].
6) Both T_3 and T_4 are biologically active, while rT_3 is biologically inactive.
7) Enzyme hydrolysis of thyroglobulin releases free T_3 and T_4 into the plasma.



Biosynthesis of thyroid hormones. (T = Tyrosine and TG = Thyroglobulin).

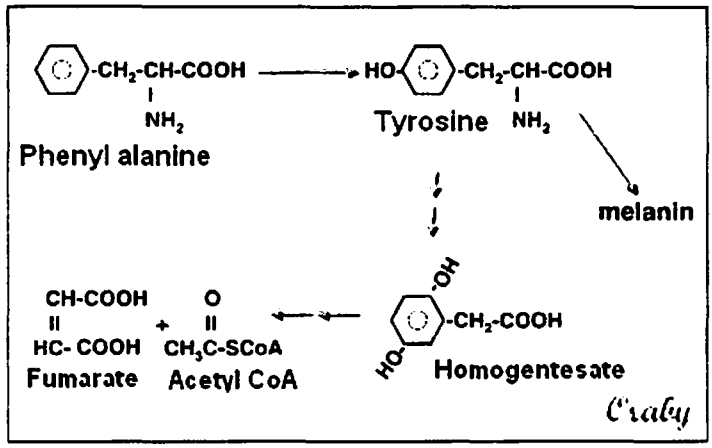
3. **Plasma T_3 and T_4:**
   a) In the plasma 99.95 % of T_3 and T_4 are transported in association with 2 proteins:
      1) **Thyroxin binding globulin** (TBG): it is the major transporter.
      2) **Thyroxin binding prealbumin.**
   b) About 0.05 % of T3 and T4 are present in a free unbound state. Free T3 and T_4 are metabolically active hormones in the plasma.
   c) T_3 is more active than T_4. It may be the only form that binds to receptors of target cells.
   d) About 2/3 of the plasma T_3 arises by deiodination of T4 in the liver.
4. **Hypothyroidism:**
   a) Resulting from insufficient amounts of free T_3 or T_4.
   b) It is usually due to thyroid failure but it can be due to disease of the pituitary or hypothalamus.
   c) The thyroid hormones insufficiency during intrauterine fetal life results in a disease shows abnormal physical development and mental retardation **(cretinism).**
   d) Measurement of T3, T4 and TSH must be done for **every newly born infant** (in the first few days after birth) as screening test of cretinism.
   e) If the disease occurs later in life, it is called: **myxodema,** which shows no mental retardation.


Cretin

5. **Hyperthyroidism (thyrotoxicosis):**
   a) It results from excessive production of thyroid hormones.
   b) Most cases are due to **Graves disease** which results from the production of antibodies that activate TSH production and in turn produces excessive amounts of T_3 and T_4. These antibodies are called: **Thyroid-stimulating IgG (TSI).**

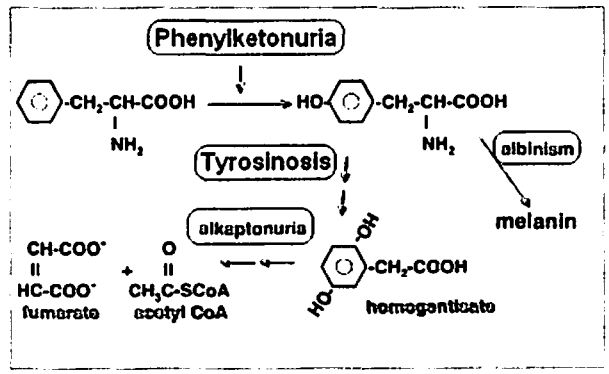
IV. *Catabolism of phenylalanine and tyrosine:*

The carbon skeletons that have left after the removal of amino groups of both phenylalanine and tyrosine may catabolize to form fumarate (glycogenic) or acetoacetate (ketogenic).



V. *Inborn errors of phenylalanine and tyrosine metabolism:*

- ***Phenylketonuria.***
- ***Tyrosinosis.***
- ***Alkaptonuria.***
- ***Albinism.***



A. <u>Phenylketonuria</u>:

1. **Definition:** It is inherited deficiency of ***phenylalanine hydroxylase enzyme***. This enzyme is present in **liver**.
   - **Atypical phenylketonuria:** It results from deficiency of ***dihydrobiopterin reductase*** enzyme.
2. **Effects (signs and symptoms):**
   a) **Mental retardation.**
   b) **Increased blood phenylalanine**: due to inability to hydroxylate phenylalanine to tyrosine. Phenylalanine is converted to phenylpyruvate and phenyllactate, which are excreted in urine.
   c) Other signs and symptoms of phenylketonuria include:
      1) Failure to walk and talk.
      2) Hyperactivity and tremors.
      3) Failure to grow.
      4) An Intelligence Quotient (IQs):

- ➢ It is a test that indicates a person's mental abilities relative to others of approximately the same age. Normally it is between 90-110.
- ➢ IQs in phenylketonuria is below 50

5) **Skin lesion e.g. eczema.**

The cause of mental retardation is unknown, but phenylpyruvate is a potent inhibitor of pyruvate translocase enzyme, which transports pyruvate to inside the mitochondria in brain cells. Pyruvate is precursor of tricarboxylic acid cycle that gives energy in brain.

3. **Frequency of phenylketonuria:**
   - The frequency of phenylketonuria is 1 in 10,000 live births.
4. **Diagnosis of phenylketonuria:**
   a) Infants are screened at birth (4^{th} day) by measuring blood phenylalanine by a test called Guthrie test, a bacterial assay for phenylalanine. Abnormal high phenylalanine level will be found in cases of phenylketonuria.
5. **Prevention of phenylketonuria:**
   a) Any infant proved to have abnormal high level of blood phenylalanine, should be fed **milk containing very low amount of phenylalanine.**
   b) This regimen of diet is maintained up to 6 years of age when a high concentration of phenylalanine has no longer effect on brain cells.

B. Hereditary tyrosinemia (tyrosinosis):

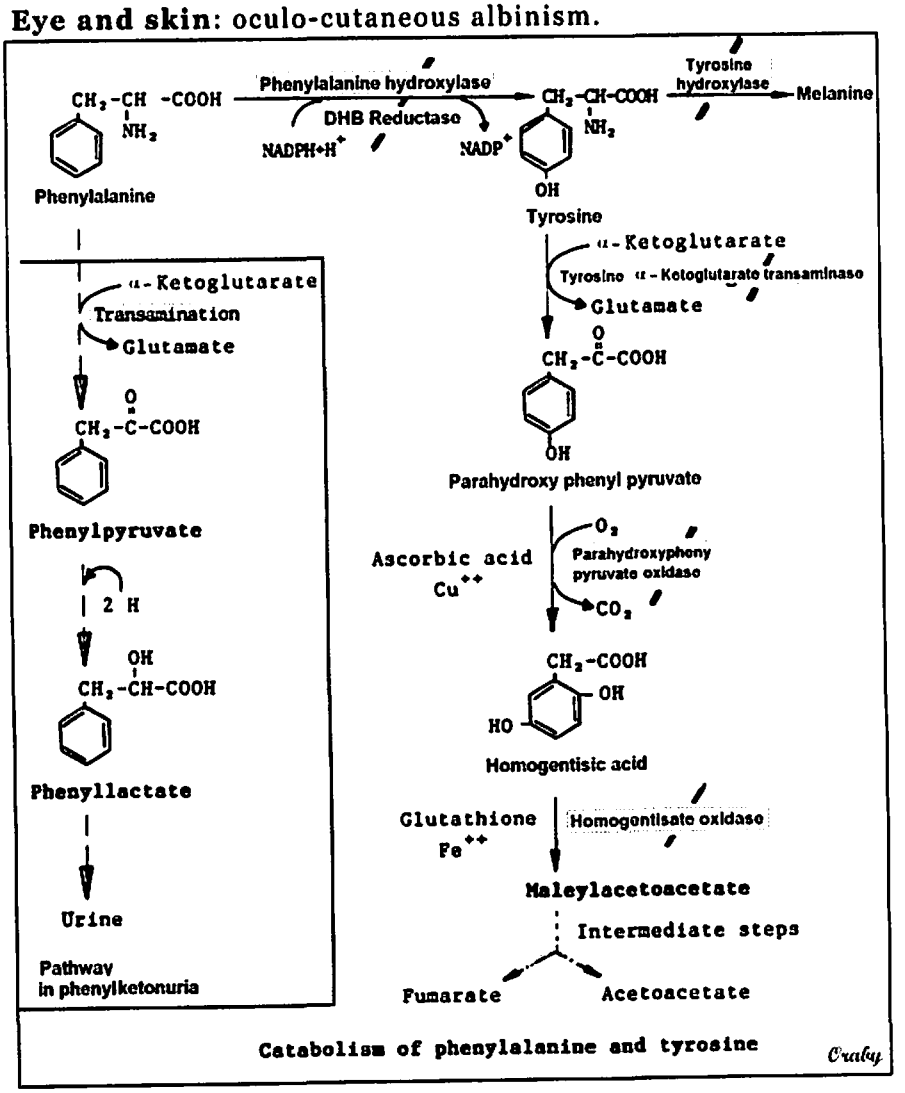
1. It is inability to metabolise tyrosine and p-hydroxy phenylpyruvate.
2. It is due to deficiency of tyrosine α-ketoglutarate transaminase and p-hydroxy-phenylpyruvate oxidase enzymes
3. **Forms of tyrosinemia:**
   a) **Acute**: where there is diarrhea, vomiting and failure to grow. Death from liver failure occurs within 7 months.
   b) **Chronic:** occurs later in life. Liver cirrhosis and hepatic carcinoma are common. There is also a mild mental retardation. For unknown cause methionine, level may be high.
4. **Prevention and treatment:**
   a) Feeding the affected infant and children a diet containing very low levels of tyrosine and phenylalanine (precursor of tyrosine).

C. **Alkaptonuria:**

1. It is a benign disease resulting from deficiency of ***homogentisate oxidase*** enzyme.
2. **Effects:** Homogentisate increases and causes:
   a) Deposition in joints causing **arthritis.**
   b) Deposition in connective tissue causing **generalized pigmentation** (ochronosis).
   c) Excreted in large amounts in urine, that is oxidized in the air giving the **dark urine (black urine when left to stand).**

D. **Albinism:**

1. It is a hereditary deficiency of **tyrosine hydroxylase** enzyme in melanocytes. This results in defective synthesis of melanin pigments. Eye, skin and hair are affected.
2. **Types of albinism:** according to the site affected:
   a) **Eye:** ocular albinism.
   b) **Skin:** cutaneous albinism.
   c) **Eye and skin:** oculo-cutaneous albinism.

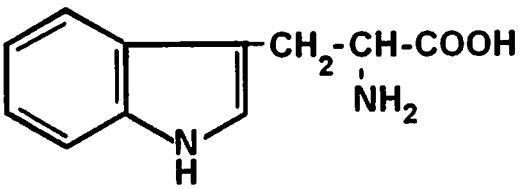


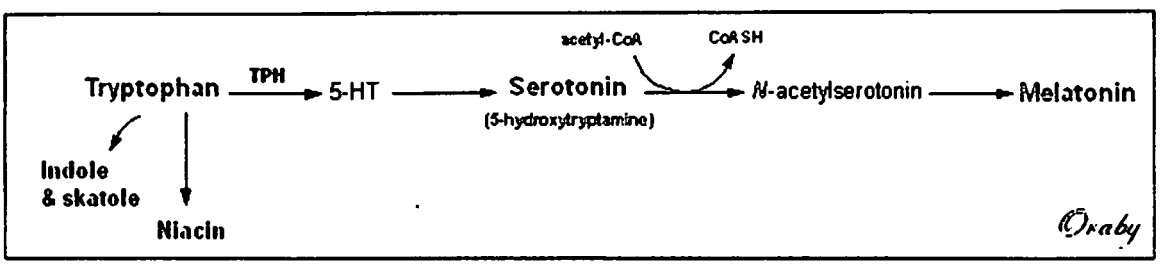
Tryptophan

I. It is glycogenic and ketogenic essential amino acid.

II. ***Functions:*** Tryptophan is the precursor of:

- ***Serotonin.***
- ***Melatonin.***
- ***Niacin (nicotinic acid)***
- ***Indole and skatole***





A. **Serotonin** also called 5-hydroxytryptamine.

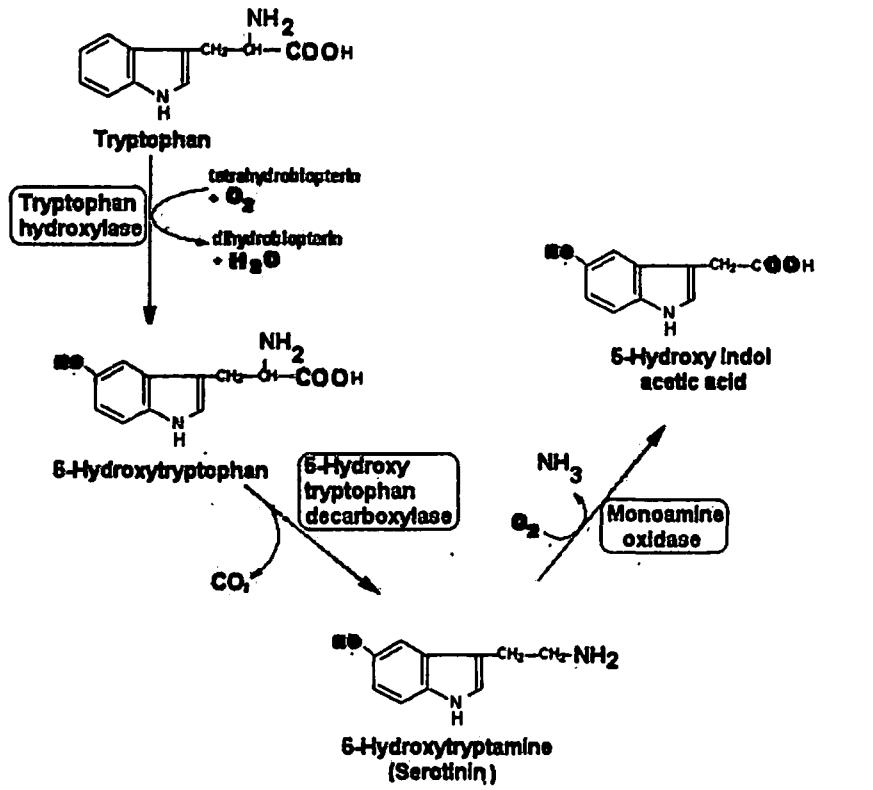
1. **Storage sites of secretion:**
   a) Hypothalamus and brain stem.
   b) Pineal gland (body).
   c) Argentaffin cells present in intestinal mucosa.
   d) Platelets.

2. **Functions of serotonin:**
   a) Neurotransmitter: it is stimulatory one.
   b) Vasoconstriction.
   c) Contraction of smooth muscle fibers.

3. **Synthesis of serotonin:**
   a) Tryptophan is hydroxylated in a reaction similar to that of phenylalanine. The product 5-hydroxytryptophan is decarboxylated to serotonin.

4. **Catabolism of serotonin:**
   a) Serotonin undergoes oxidative deamination by monoamine oxidase **(MAO).** The resulting compound, **5-hydroxyindol acetic acid** is excreted in urine.
   b) Certain substances can inhibit MAO enzyme e.g. iproniazide drugs. This causes increase of serotonin, a stimulatory

transmitter in brain. These drugs are used in treatment of psychic conditions as depression.



Synthesis and catabolism of serotinin. *Oraby*

Argentaffinoma (carcinoid syndrome):

**It is a malignant disease characterized by excessive production of serotonin by argentaffin cells of intestinal mucosa.*

**This occurs on the expense of niacin synthesis. Thus symptoms and signs of pellagra developed.*

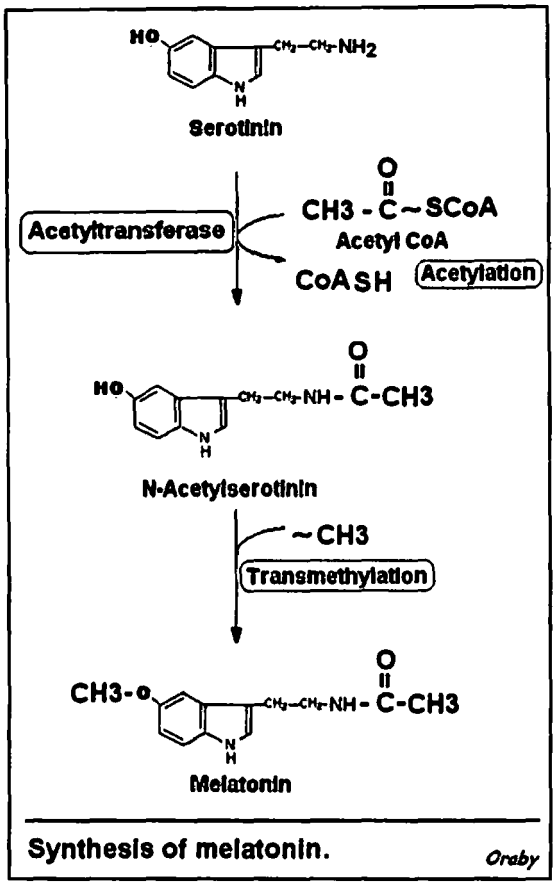
**It causes also diarrhea and broncho-spasm.*

**Urinary excretion of 5-hydroxyindol acetic acid is increased.*

B. Melatonin:

1. **Site of secretion:** pineal gland
2. **Functions of melatonin:**
   a) It inhibits gonadal functions.
   b) It has sleep inducing effect.
   c) It inhibits synthesis and secretion of other neurotransmitters such as dopamine and GABA.
   d) Regulation of circadian rhythm, being synthesized mostly at night.

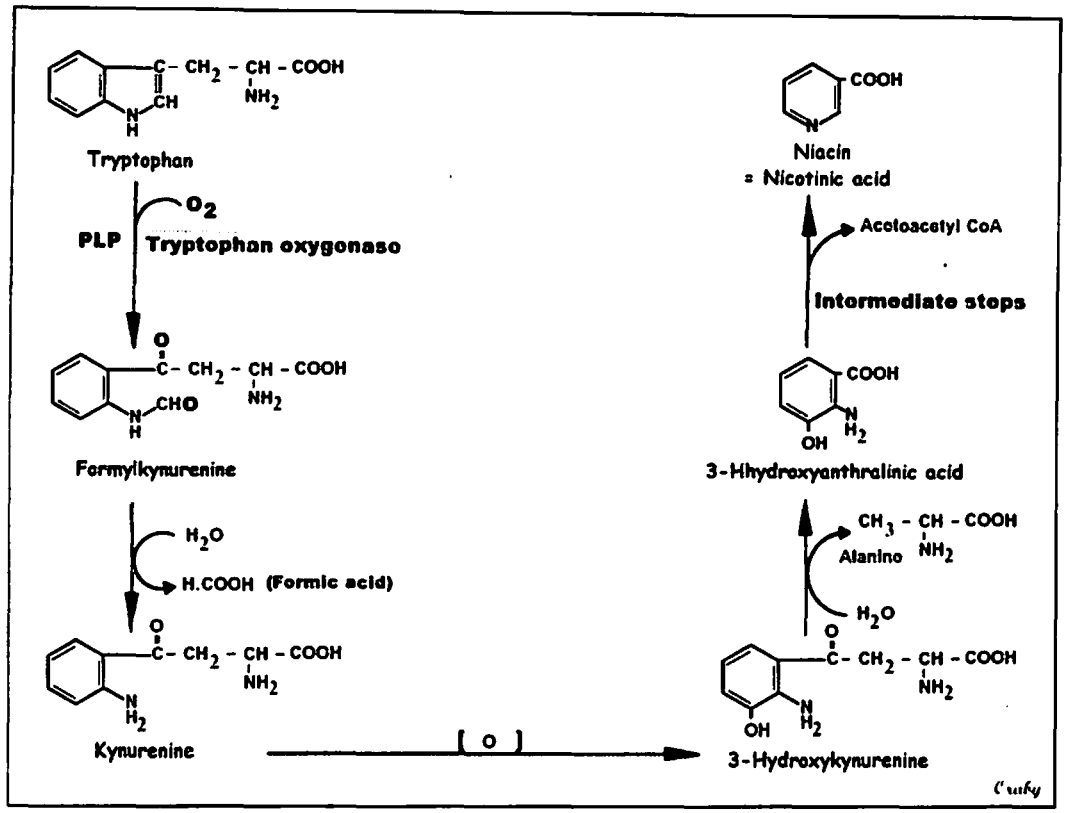
3. **Synthesis of melatonin:**
   a) It is synthesized in pineal gland, as the **acetyltransferase** needed for melatonin synthesis is present in pineal gland. Melatonin is synthesized from serotonin by acetylation followed by methylation.
   b) Melatonin is secreted only at night (dark). This is because the release of melatonin is inhibited by light entering the eye and transmitted to the pineal gland by way of the CNS.



Synthesis of melatonin. *Oraby*

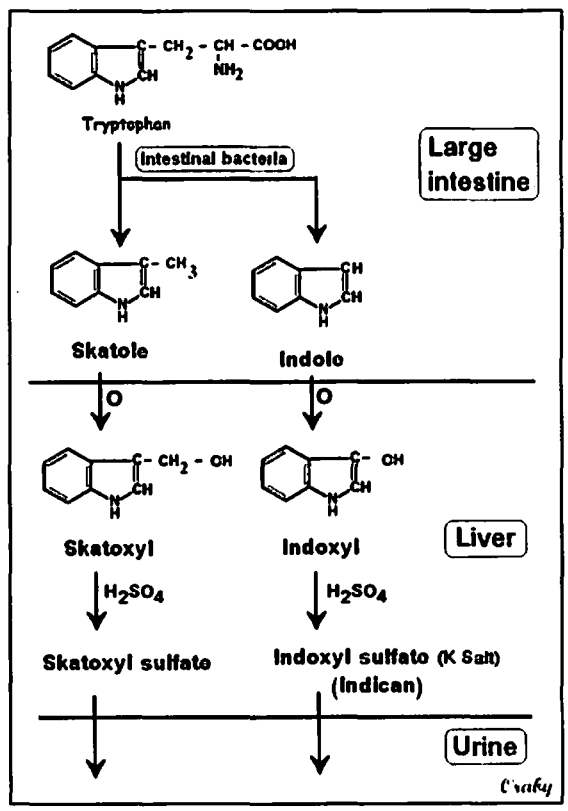
C. **Niacin (nicotinic acid):**

1. **Functions of niacin:**
   Niacin is a member of vitamin B complex, being synthesized in liver.
   a) It is essential for synthesis of NAD^+ and $NADP^+$ coenzymes, which act as hydrogen carriers in varieties of metabolic reactions.
   b) Niacin lowers plasma cholesterol. This is because it inhibits the flow of free fatty acids (FFA) from adipose tissue, which provides acetyl CoA essential for cholesterol synthesis.
2. **Synthesis of niacin:**
   a) It is synthesized in the course of tryptophan catabolism.
   b) Every 60 mg tryptophan are converted into 1 mg niacin.
3. **Pellagra:**
   a) This is a disease resulting from deficiency of niacin formation.
   b) It results from deficiency of either niacin tryptophan, or pyridoxine.
   c) It is the disease of 3 Ds. These are diarrhea, dermatitis and dementia.



D. Indole and skatole:

1. These are putrefactive products of tryptophan produced by bacteria in large intestine.
2. Indol and skatol give the characteristic odor of stool.
3. Indole and skatole may be absorbed and go to the liver to be hydroxylated and conjugated with sulphate and excreted in urine as salt forms: potassium skatolxyl sulphate and potassium indoxyl sulphate (=indican).



Hartnup`s disease:

1-It is a hereditary abnormality in tryptophan metabolism where the intestinal absorption and renal tubular reabsorption of this amino acid are impaired.

2-It is characterized by ***pellagra skin rashes****, psychiatric changes and mental retardation.*

3-There is excess excretion of tryptophan together with lysine and histidine in urine (aminoaciduria).

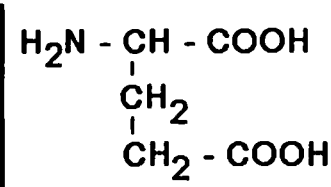
4-Adminstration of nicotinamide usually relieves all symptoms except aminoaciduria.

Glutamic acid

I. **Glutamic acid is a nonessential glycogenic amino acid.**

II. ***Functions of glutamic acid:***

- *Removal of amino group of most amino acids.*
- *Glutathione synthesis*
- *Glutamine synthesis*
- *Enzyme activator.*
- *Folic acid synthesis.*
- *Neurotransmitter.*
- *Gamma aminobutyric acid (GABA).*



A. **<u>Removal of amino group</u>** of most amino acids in the form of ammonia through transdeamination (see deamination of amino acids)

B. **<u>Glutathione synthesis</u>:**

This is a tripeptide forming of three amino acids: glutamate, cysteine and glycine (see glycine metabolism).

C. **<u>Glutamine synthesis</u>:**

Glutamine is synthesized from glutamate in the reaction catalyzed by glutamine synthetase (for functions of glutamine, see fate of ammonia).

D. **<u>Enzyme activator</u>:**

N-Acetylglutamate activates carbamoyl phosphate synthase I enzyme (see urea biosynthesis).

E. **<u>Folic acid synthesis</u>:**

1. Folic acid is a member of vitamin B-complex being composed of pteridine base, Para-amino benzoic acid (PABA), and one or more glutamic acid residues.

2. **Folic acid acts as a carrier of one carbon units, which has important role in amino acid metabolism and purine and pyrimidine synthesis (see water soluble vitamins, part I).**

F. **Neurotransmitter:**

Glutamate acts as excitatory neurotransmitter in all CNS neurons.

G. **Gamma aminobutyric acid (GABA):**

1. **Functions of GABA:**

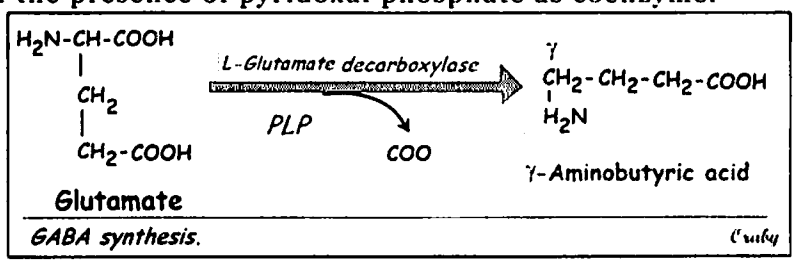
It is an inhibitory transmitter in brain and spinal cord.

a) In the brain, GABA is a postsynaptic inhibitory transmitter.

b) In the spinal cord, GABA is pre-synaptic inhibitory transmitter.

2. **Synthesis of GABA:**

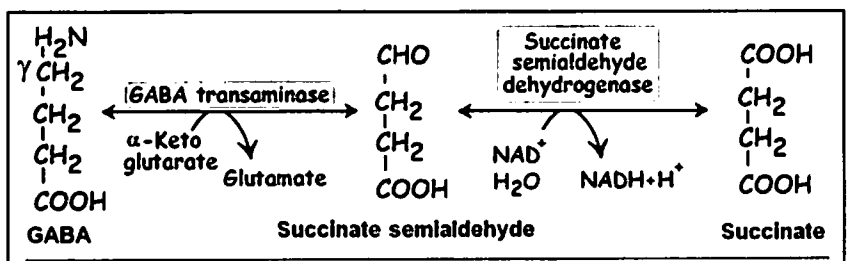
It is synthesized from L-glutamate by L-glutamate decarboxylase in the presence of pyridoxal phosphate as coenzyme.



GABA synthesis.

3. **Catabolism of GABA:**

It is metabolized within the neurons to succinate:



Catabolism of GABA.

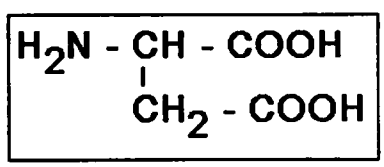
4. **Deficiency of GABA:**

a) It may be due to deficiency of either: L-glutamate decarboxylase or pyridoxal phosphate.

b) It causes convulsions especially in children.

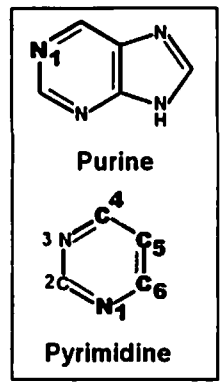
Aspartate

I. It is non-essential glycogenic amino acid.



II. *Functions of aspartate:*

- ***Urea formation***
- ***Purine formation***
- ***Pyrimidine formation***
- ***Asparagine***
- ***Neurotransmitter***
- ***β-Alanine***



A. **Urea formation:**

Aspartate reacts with citrulline to form arginosuccinate.

B. **Purine formation:**

Aspartate is the source of N_1 of purine.

C. **Pyrimidine formation:**

Aspartate is the source of N_1, C_4, C_5 and C_6 of pyrimidine.

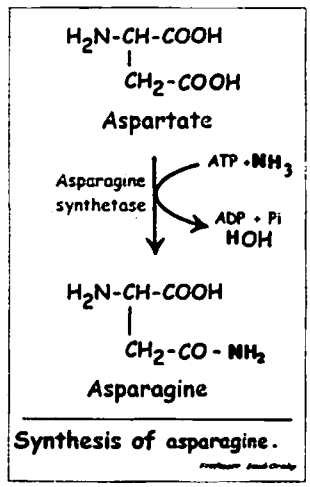
D. **Asparagine formation:**

1. **Functions:**

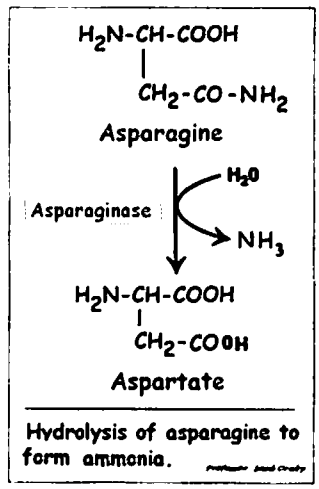
   a) It enters in the structure of some proteins e.g. **oxytocin hormone.**

   b) It is a source of **ammonia** especially in plants.

2. **Synthesis and breakdown:**



Synthesis of asparagine.



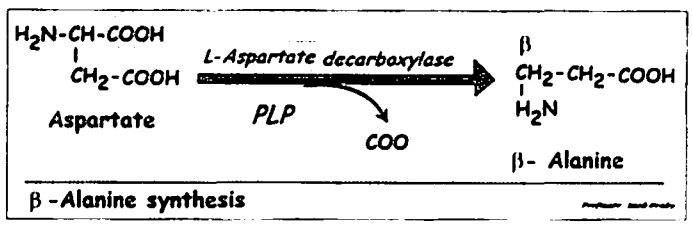
Hydrolysis of asparagine to form ammonia.

E. **Neurotransmitter:**

Aspartate acts as an excitatory transmitter on all CNS neurons.

F. **β-Alanine:**

1. In bacteria, decarboxylation of aspartic acid produces β-Alanine.

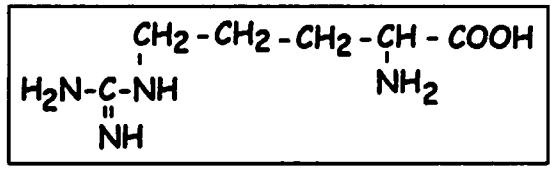


β -Alanine synthesis

2. In mammalian tissues β alanine arises during catabolism of cytosine base.
3. β-Alanine enters in the structure of: pantothenic acid, coenzyme A, carnosine, anserine and homocarnosine.

Arginine

I. It is glycogenic, semi-essential amino acid i.e. formed in amount not sufficient for body especially in children and pregnant females.



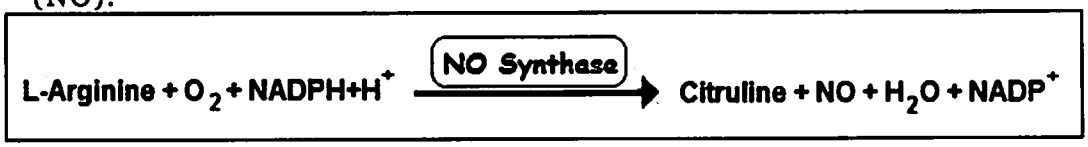
II. *Functions of arginine:*

A. **Urea formation:** see urea cycle.

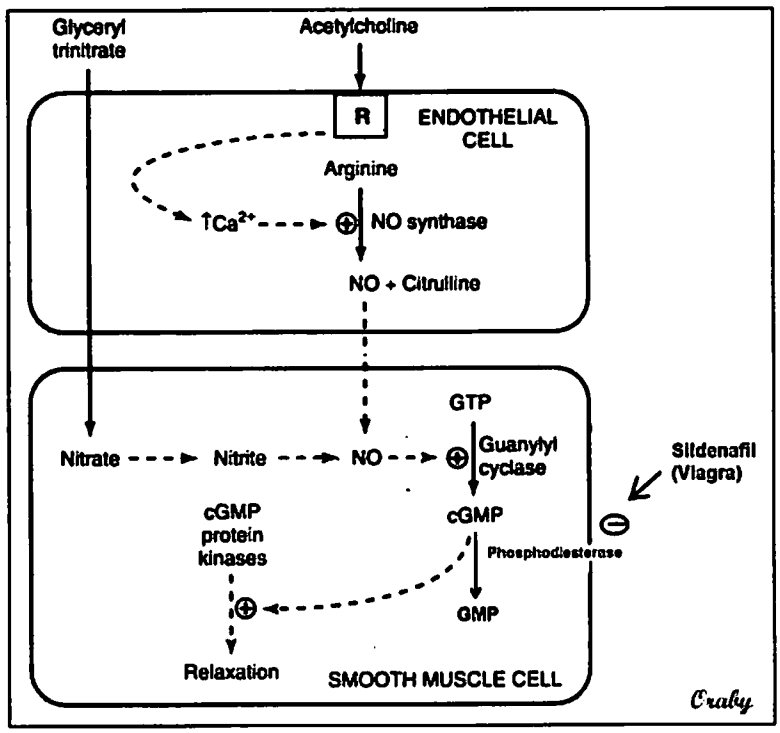
B. **Creatine formation:** see glycine metabolism.

C. **Arginine phosphate** (arginine ~ P): it is present in muscles and acts as a source of energy in animals (invertebrate).

D. **Nitric oxide:** L-Arginine serves as a precursor of nitric oxide (NO).



1. NO is an intracellular signalling molecule and functions as neurotransmitter, smooth muscle relaxant and vasodilator (through cGMP).
   a) **NO synthase** is a complex enzyme using 5 cofactors: NAD, FAD, FMN, heme and tetra-hydrobiopterin

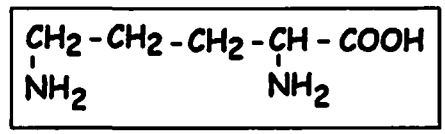


2. Nitroglycerine (glyceryl trinitrate) is a powerful coronary vasodilator through increasing NO formation.

3. Sildenafil **(Viagra)** is a drug that inhibits phosphodiesterase enzyme that converts cGMP into GMP. This maintains the action of cGMP as smooth muscle relaxant and vasodilator and used as a drug that maintains penile erection.

Ornithine

I. It is a glycogenic, non-essential amino acid.



II. ***Functions:*** Ornithine is important for:

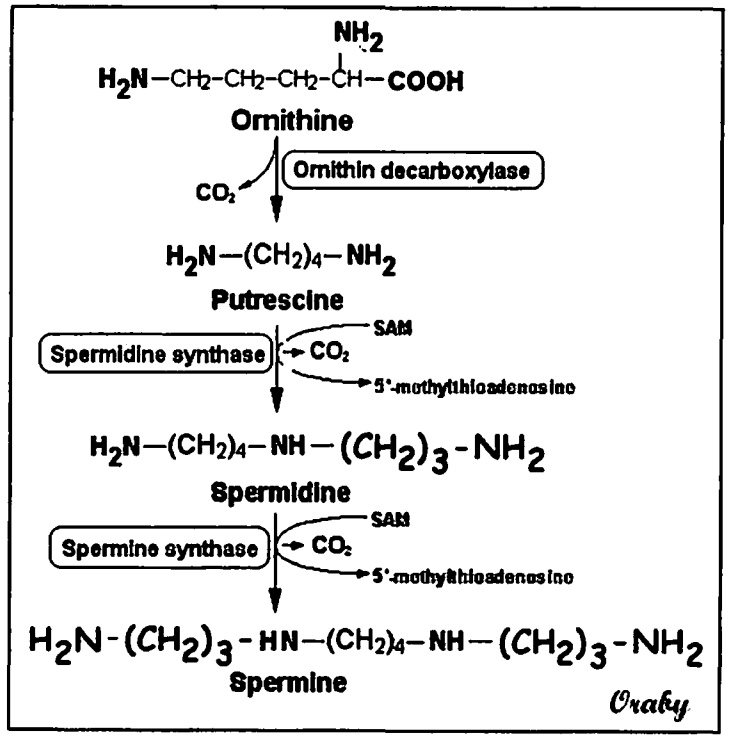
A. **Urea formation.**

B. **Detoxication:**

Ornithine + Phenyl acetyl CoA (Toxic) → Phenyl acetyl ornithine (non-toxic)

C. **Spermidine and spermine formation:** These are polyamines formed in prostate by ornithine and methionine.

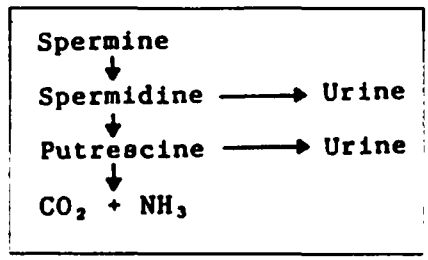
1. **Functions:**
   a) Cell proliferation (division) and growth.
   b) Stabilization of intact cells, cell membrane and sub-cellular organelles.
   c) They give the characteristic odor of semen.
   d) Stimulation of DNA and RNA biosynthesis.
   e) Inhibition of protein kinase enzyme.
   f) In pharmacological doses, polyamines cause decrease in both blood pressure (hypotension) and body temperature (hypothermia).
   g) They have antibacterial action.
2. **Biosynthesis:** See the figure.



3. Catabolism:

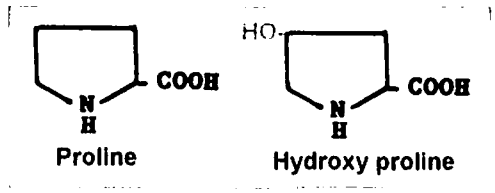
a) Spermine is oxidized to spermidine and putrescine.

b) Both are either excreted in urine or converted to CO_2 and NH_3.



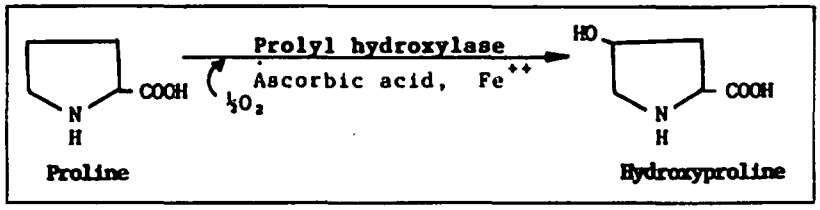
Proline and hydroxyproline

I. These are non-essential glycogenic imino acids.



II. *Functions of proline:*

A. Hydroxyproline synthesis:

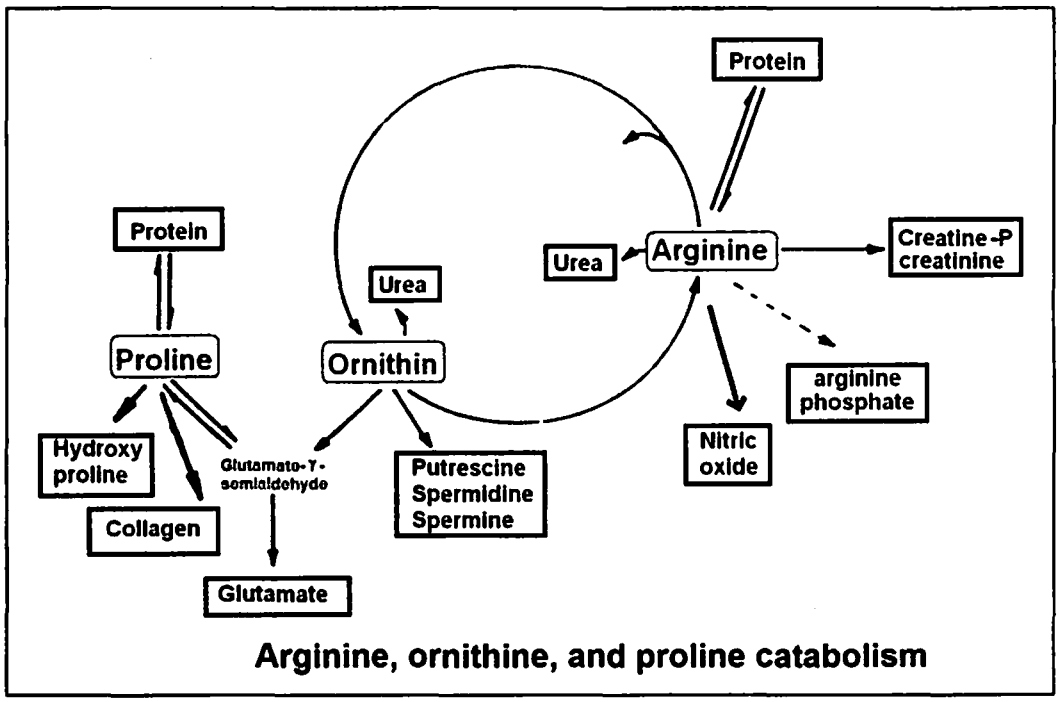


B. Collagen synthesis:

1. Proline and hydroxyproline are very rich in collagen.
2. Ascorbic acid deficiency leads to a weak collagen (scurvy).

III. *Catabolism of proline:*

gives glutamate.



Arginine, ornithine, and proline catabolism

Sulfur containing amino acids

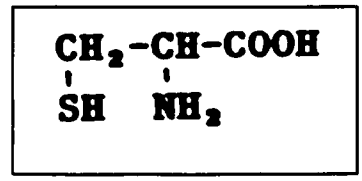
Cysteine
Cystine
Methionine

Cysteine

I. Cysteine is glycogenic, non-essential amino acid.

II. ***Functions*:** It enters in the synthesis of:

- *Glutathione*
- *Taurine*
- *Thioethylamine*
- *Proteins*
- *Detoxication*



A. **Glutathione:**

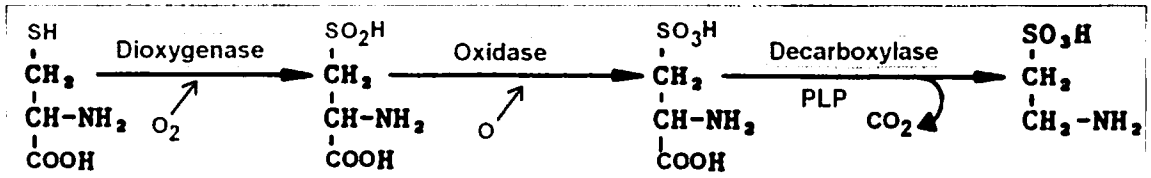
Discussed in glycine metabolism.

B. **Taurine:**

1. **Functions:**

It combines with cholic acid to form taurocholic acid. Its sodium salt (sodium taurocholate) is one of bile salts, which are important for digestion, and absorption of lipids.

2. **Synthesis:**



Synthesis of taurine.

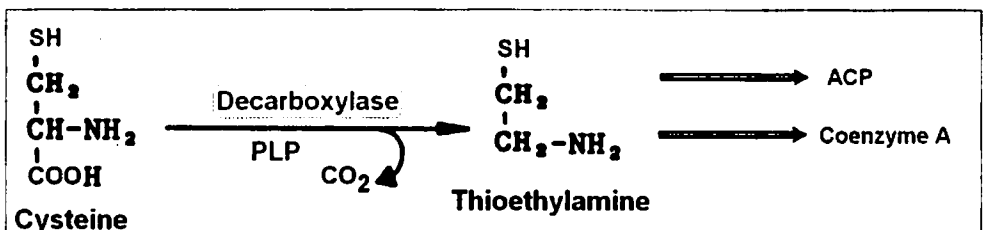
C. **Thioethylamine:** A part of the vitamin: Pantothenic acid.

1. **Functions:** it enters in the structure of:

a) **Coenzyme A:** It is a coenzyme, which is important in carbohydrates and lipids metabolism.

b) **Acyl carrier protein(ACP):** This is a component of fatty acid synthase enzyme.

2. **Synthesis:**

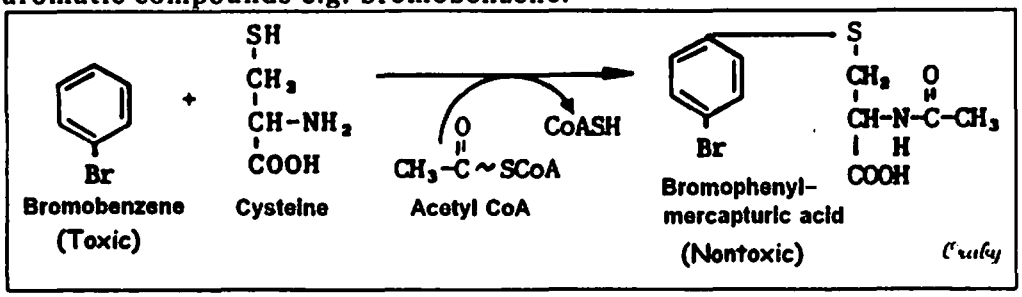


Synthesis of thioethylamine.

D. **Protein synthesis:** Cysteine is very important amino acid for some proteins as:

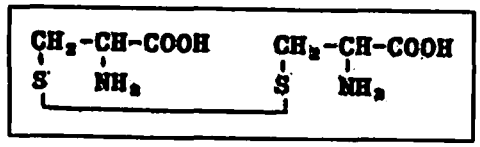
1. **Keratins:** simple proteins that are present in hair , nail , skin etc. Keratins are very rich in cysteine.
2. **Many enzymes:** e.g. glyceraldhyde-3-phosphate dehydrogenase contains in its active center -SH group derived from cysteine.

E. **Detoxication:** Cysteine is important for the detoxication of some aromatic compounds e.g. bromobenzene.



Cystine

I. Cystine (di-cysteine) is glycogenic, non-essential amino acid.



II. ***Functions:***

A. **Protein structure:** The -S-S- group of cystine is important for tertiary and quaternary structure of proteins e.g. insulin hormone has two -S-S- groups.

B. **Cysteine formation:**

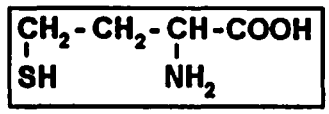
Cystinuria (cystine - lysinuria):

* It is a hereditary disease characterized by amino aciduria (excessive excretion of cystine together with basic amino acids; lysine, arginine, and ornithine.

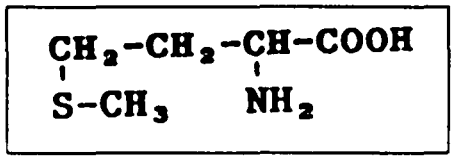
**It is due to defective renal tubular reabsorption of these 4 amino acids. Cystine may precipitated in renal tubules forming renal stones.*

Homocysteine

A homocysteine test measures the amount of the amino acid homocysteine in the blood. Homocysteine may get high levels when cholesterol, white blood cells, calcium, and other substances (plaque) build up in blood vessels. This build-up may lead to a heart attack, stroke, and blood clots in the lungs (pulmonary embolism) or deep veins of the legs (deep venous thrombosis).



Methionine



I. Methionine is glycogenic, essential amino acid.

II. ***Function:*** It enters in the synthesis of:

A. **S-Adenosylmethionine (SAM):** The main methyl donor, which used in transmethylation reactions.

B. **Cysteine synthesis:** Through formation of homocysteine, this reacts with serine to give cysteine.

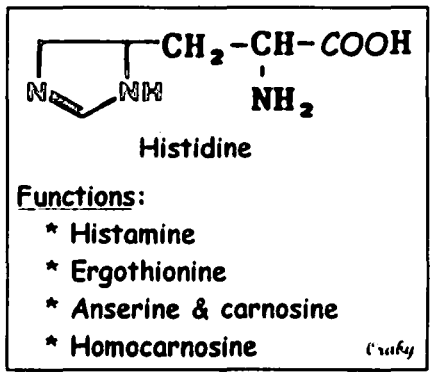
C. **Lipotropic factor:** Methionine is one of lipotropic factors, which prevent fatty liver.

D. **Spermidine and spermine:** see ornithine metabolism.

Histidine

I. It is essential, glycogenic amino acid.

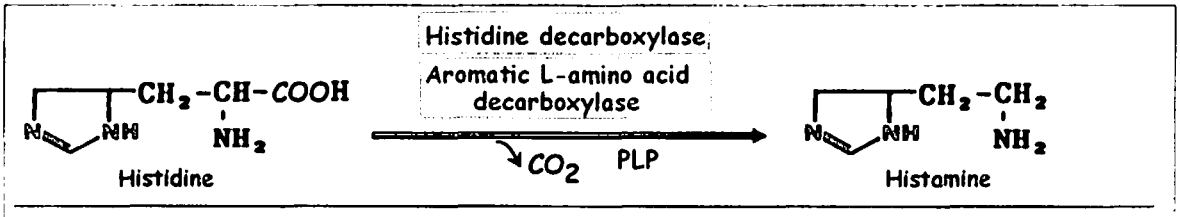
II. ***It is used for synthesis of:***



A. **Histamine:**

1. **Functions:** Histamine is secreted by mast cells as a result of allergic reactions or trauma. It has the following functions:
   a) Vasodilatation.
   b) Contraction of smooth muscles of bronchi.
   c) Stimulation of gastric secretions.
   d) Histamine also acts as **excitatory neurotransmitter.** These are large amounts of histamine in the hypothalamus and in anterior and posterior lobes of the pituitary gland.

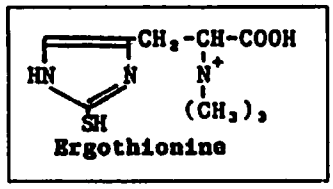
2. **Synthesis:**
   Histamine is derived from histidine by decarboxylation. This reaction is catalyzed by either 2 different enzymes:
   a) Aromatic L-amino acid decarboxylase or
   b) Histidine decarboxylase.



Synthesis of histamine.

B. **Ergothionine:**

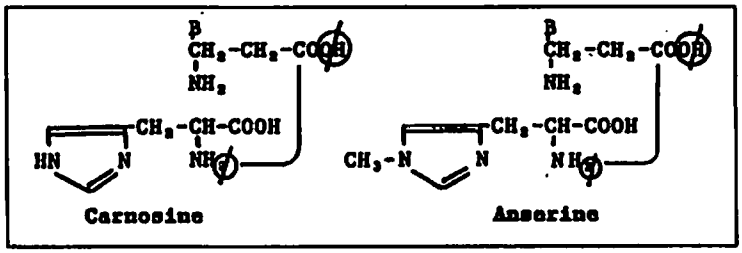
1. It is N-trimethyl, thiol histidine.
2. *Ergothionine* is an intracellular antioxidant naturally occurring in plants and animals.



Ergothionine

C. **Carnosine and Anserine:**

1. Carnosine is a substance results from conjugation of histidine with β-alanine.
2. Anserine is produced by methylation of carnosine.
3. Both are present in skeletal muscles and not in cardiac muscle.
4. **Functions:**
   a) Anserine and carnosine activate myosin ATPase enzyme.
   b) They have antioxidant activity and have a role in copper metabolism.



D. **Homocarnosine:**

1. It is a dipeptide composed of the amino acid derivative, Gamma-Amino Butyric Acid (GABA) and L-histidine.
2. It is present in brain at levels 100 times higher than carnosine levels.
3. Homocarnosine acts as nervous system dipeptide (neuropeptide).

Serine

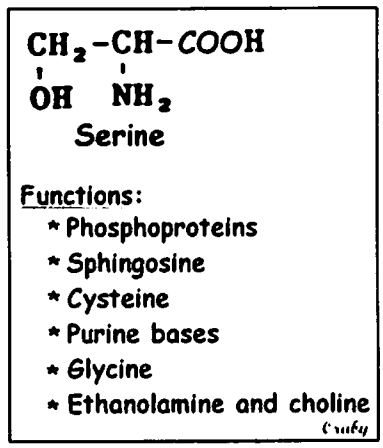
I. Serine is non-essential, glycogenic amino acid.

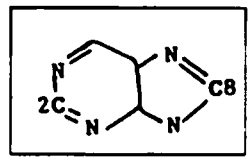
II. ***Serine is used for the synthesis of:***

A. **Phosphoproteins:** The -OH group of serine residues present in proteins is the site of esterification of phosphate to form phosphoproteins.

B. **Sphingosine base:** Serine reacts with palmityl CoA to form sphingosine base. It enters in the structure of sphingomyelin (see lipid metabolism part II).

C. **Cysteine:** Serine reacts with homocysteine (derived from S-adenosyl methionine) to form cysteine (see methionine catabolism).

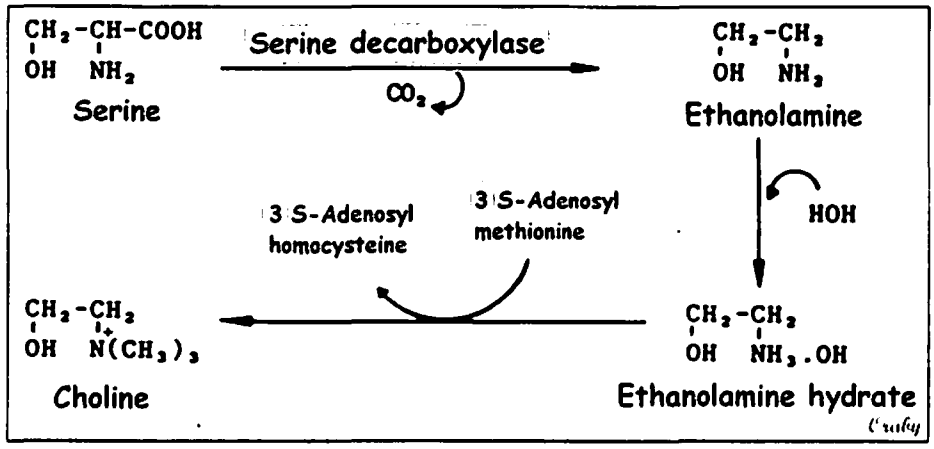




D. **Purine bases:** β-carbon of serine enters in the formation of C_2 and C_8 of purine bases.

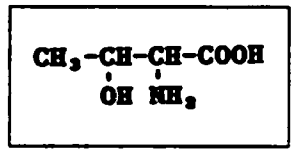
E. **Glycine:** Through the action of serine hydroxy methyl transferase.

F. **Ethanolamine and choline:** Serine, ethanolamine and choline are important constituents of phospholipids.



Threonine

I. It is essential, glycogenic amino acid.



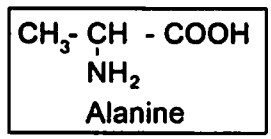
II. ***Threonine is used for the synthesis of:***

A. **Phosphoproteins:** Like serine, the -OH group of threonine is important for the synthesis of phospho-proteins.

B. **Glycine:** Through the action of threonine aldolase enzyme.

Alanine and β-alanine

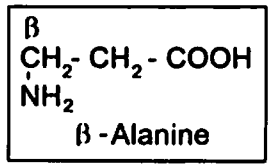
I. These are non-essential, glycogenic amino acid.



II. ***Alanine is important for:***

A. Alanine -together with glycine- forms a major fraction of plasma amino acids.

B. Alanine is the main amino acid that is converted into glucose in liver through alanine-glucose cycle.



C. Alanine is a major component of bacterial cell wall.

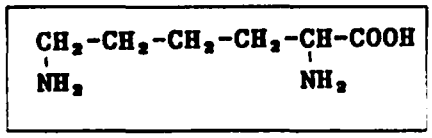
III. ***β-Alanine is important for the synthesis of:***

A. Pantothenic acid, anserine and carnosine.

B. β-Alanine **does not** enter in the formation of proteins.

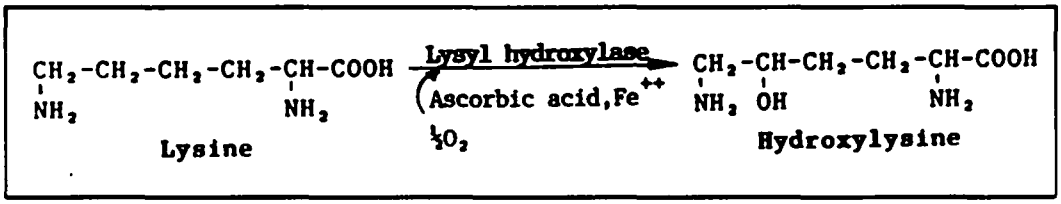
Lysine and hydroxylysine

I. Lysine is an essential, glycogenic amino acid.



II. ***Lysine is important for:***

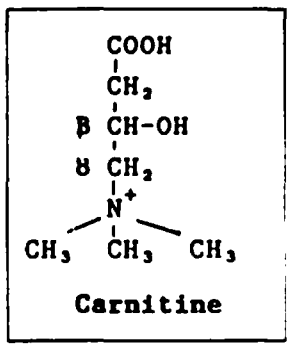
A. **Hydroxylysine synthesis:**



B. **Collagen synthesis:** Lysine and hydroxylysine are very rich in collagen.

C. **Carnitine synthesis:**

1. It is β-hydroxy, γ-trimethyl amino butyric acid.
2. It is synthesized from lysine, at first by methylation (using S-adenosyl methionine), then deamination and finally by removal of 2 carbons to give carnitine.
3. **Functions:** Carnitine acts as a carrier, transporting long chain acyl CoA across inner mitochondrial membrane. This is essential for fatty acid oxidation (see lipid metabolism, part II).



Branched chain amino acids
Valine - Leucine - Isoleucine

1. All are essential amino acids.
2. Valine is glycogenic, leucine is ketogenic and isoleucine is glycogenic and ketogenic.
3. All enter in the formation of body proteins.
4. Catabolism of branched chain amino acids are discussed before.

Protein turnover

1. Most proteins in the body - with the exception of collagen - are in constant state of degradation and synthesis.
2. In healthy adult, the rate of protein synthesis is equal to that degraded (about 125 to 220 grams/day). Thus the total amount of protein in the body remains constant. This process is called **protein turnover.**
3. In certain conditions as during growth, the rate of protein synthesis is greater than degradation. This is to provide body needs of proteins.

Nitrogen balance

I. ***Nitrogen balance*** means that nitrogen intake is equal to nitrogen loss from the body.

A. **Nitrogen intake:** Nitrogen is taken in the form of dietary proteins. Every 100 gm protein contain 16 gm nitrogen:

B. **Nitrogen loss:** Nitrogen is lost from the body through non-protein nitrogenous (NPN) compounds:

1. **In urine:**

Urea (main solute)	20-40	g/day
Uric acid	0.5	g/day
Hippuric acid	0.7	g/day
Ammonia	0.7	g/day
Creatinine	0.7-1.7	g/day
Creatine	0-0.2	g/day
Amino acids	100	mg/day

2. **In feces:** One gram / day is excreted in feces.
3. **In milk** and menstrual fluids in female.

II. ***Positive and negative nitrogen balance:***

A. **Positive nitrogen balance:** It means that nitrogen intake is greater than nitrogen loss. It occurs in conditions where the formation of tissue proteins is increased e.g. growing children and muscle training.

B. **Negative nitrogen balance:** It means that nitrogen intake is less than nitrogen loss. It occurs in conditions where breakdown of tissue proteins is increased e.g. diabetes mellitus and starvation, hyperthyroidism, T.B. and aging.

Diseases related to protein metabolism

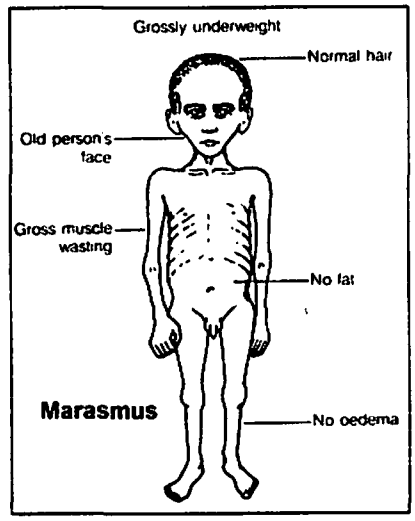
I. ***Hypoproteinosis***

A. **Definition:** This condition is due to dietary deficiency of proteins.

B. **Effects:**

1. **In adult:** This leads to hypoproteinemia with subsequent edema, weakness of muscles, anemia and increased susceptibility to infections.

2. **In infants:**
   a) **Kwashiorkor:** This is a disease resulting from deficiency of dietary protein only. It leads to growth retardation, anemia, vomiting and anorexia (loss of appetite).
   b) **Marsmus:** this is a disease resulting from deficiency of dietary protein together with dietary carbohydrate and fat.



II. ***Neurologic disorders results from abnormalities in protein structure:***

A. **Alzheimer's disease:**

1. It is a neurological disorder resulting from deposition of an insoluble protein known as β-amyloid in brain and nervous tissue.
2. The disease is characterized by dementia, declining activities of daily living and by neuropsychiatric symptoms or behavioral changes.

B. **Spongiform encephalopathies (prion disease or mad cow disease):**

1. It is a neuro degenerative disease due to aggregation and deposition of insoluble protein known as prion protein.
2. Prion diseases affect the nervous system in humans and animals.
3. In people, prion diseases impair brain function, causing memory changes, personality changes, dementia, and problems with movement that worsen over time. It ends with death.

Role of liver in protein metabolism

1. ***Removal of amino group*** from amino acids by deamination, transamination or transdeamination.
2. Synthesis of ***urea*** from ammonia.
3. Synthesis of amino acid derivatives e.g. ***creatine***, taurine ec.
4. Synthesis of ***plasma proteins*** and coagulation factors.
5. Synthesis of ***non-essential amino acids***.
6. Catabolism of ***carbon skeletons of amino acids*** and formation of glucose (gluconeogenesis) or ketones (ketogenesis).
7. ***Detoxication*** of many toxic compounds by conjugation with some amino acids e.g. glycine, glutamine.

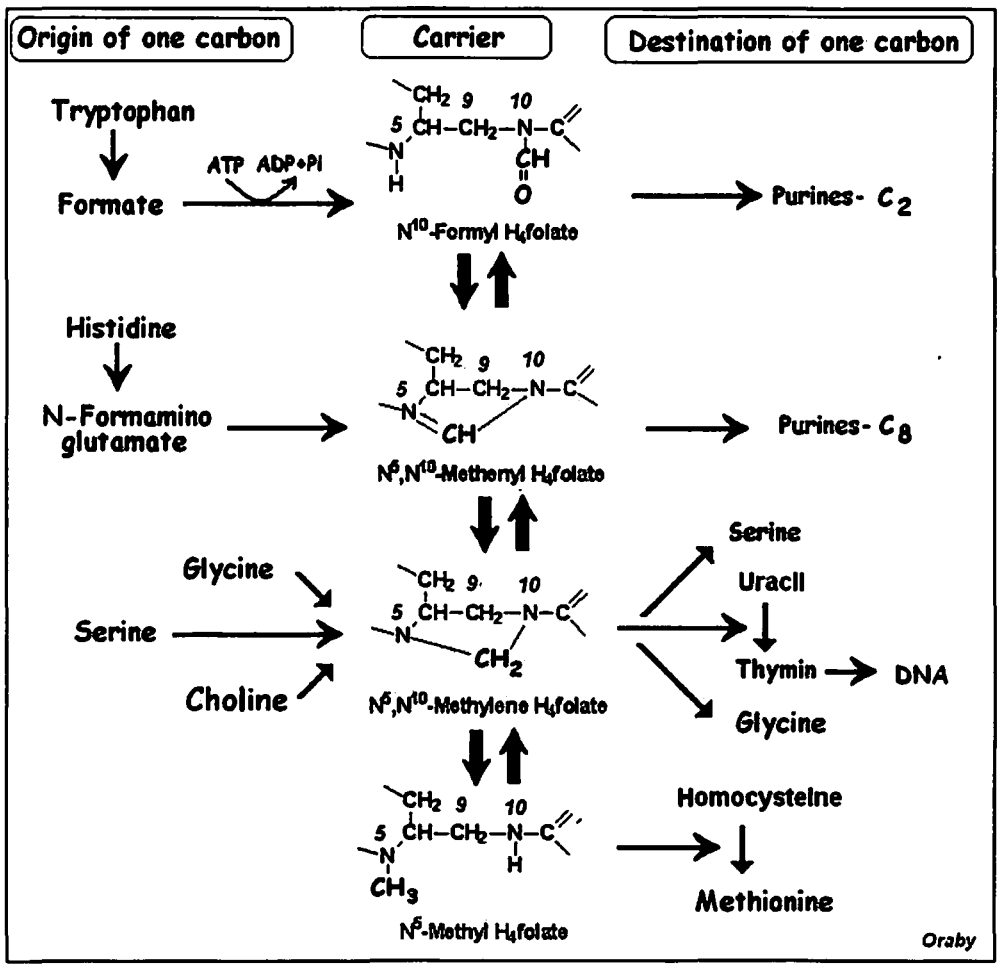
Hormones that regulate protein metabolism

1. **Anabolic hormones:** Insulin, growth hormone and androgens are anabolic hormones i.e. stimulate protein biosynthesis.

2. **Catabolic hormones: Glucocorticoids** are catabolic hormones i.e. inhibit protein biosynthesis and stimulate gluconeogenesis.
3. **Thyroid hormones:** are anabolic in physiological doses and catabolic in large doses.

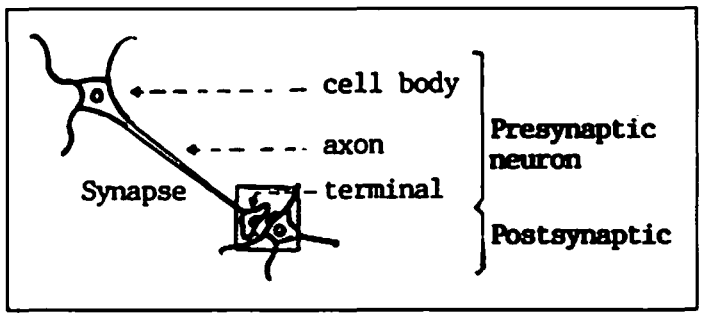
Metabolism of one carbon fragment (Role of folic acid in amino acid metabolism)

1. The following diagram shows the summary of one carbon metabolism.
2. **Source of one carbon:** Many amino acids are sources of one carbon that is carried by folic acid to be utilized in varieties of metabolic reactions: serine, tryptophan and histidine.
3. For detail, see part I, chapter of vitamins.



Neurotransmitters

I. Introduction:



A. **Definition:**
Neurotransmitter is a chemical substance synthesized and released by one neuron (by presynaptic terminal) onto a specific receptor or an adjacent cell (postsynaptic).

B. Most of neurotransmitters are amino acids, amino acid derivatives, or peptides.

C. Neurotransmitter is released upon nerve stimulations to initiate the activity of postsynaptic cell.

II. Excitatory and inhibitory transmitters:

A. **Excitatory:** as acetylcholine, catecholamines, serotonin, glutamate, aspartate and histamine.

B. **Inhibitory:** as glycine and GABA.

III. Classification:

- ***Amino acids:*** *Glycine, glutamate and aspartate.*
- ***Amino acid derivatives:*** *Catecholamines, serotonin, GABA, and histamine (discussed before).*
- ***Neuropeptides:*** *They include substance P, endogenous opioid peptides, somatostatin, thyrotropin releasing hormone (TRH), corticotropin releasing hormone, vasoactive intestinal polypeptide, cholycystokinin and neurotensin.*
- ***Acetyl choline.***

A. **Neuropeptides:**

1. **Substance P:**
   a) It is present in the ending of primary sensory neurons of the spinal cord and brain stem.
   b) It may be an excitatory transmitter of pain impulses.
2. **Endogenous opioid peptides:**
   a) These naturally occurring peptides act on opiate receptors in the brain.

b) They give morphine like action and control transmission along pain pathway.

c) They include:

1) **Endorphins:**
   - These are large peptides present mainly in the pituitary and hypothalamus.
   - Three types are present α , β and γ . The most potent is β-endorphins, which is 18-30 times more potent than morphine.
   - Endorphins inhibit the release of substance P i.e. relieve pain.

2) **Enkephalins:** These are two endogenous pentapeptides isolated from brain tissue.

3) **Dynorphins:** They are found in submucous gut plexus, nervous tissue and posterior pituitary gland.

B. **Acetylcholine:** It is released at the motor end plate. It combines with nicotinic acetylcholine receptors on the postsynaptic muscle membrane, transmitting the nerve impulse.

1. **Synthesis:** By condensation of choline and acetyl CoA

Choline + Acetyl CoA —*Cholinyl transferase*→ Acetylcholine

2. **Degradation:** Acetylcholine is degraded by the enzyme acetylcholine esterase at the postsynaptic membrane.

Myasthenia gravis:

* *This is an autoimmune disease characterized by muscleweakness.*
* *It is due to defective neuromuscular transmission as a result of ↓ acetylcholine action.*
* *There are antibodies to the nicotinic acetylcholine receptors ➔ Inhibition of these receptors.*

IV. *Parkinson's disease:*

A. **Causes:** Due to decrease of dopamine neurotransmitter in mid brain ➔ *(gradual degeneration of certain cerebral area of substania nigra present in mid brain).*

B. **Signs and symptoms:** It occurs over 60 years. It includes static tremors (pills rolling). These tremors interfere with motor functions of skeletal muscles.

Summary of genetic defects in amino acids metabolism:

Amino acid	Disease	Disorder (or affected enzyme)	Signs and Symptoms	Remarks
Phenyl-alanine and tyrosine	Phenylketonuria	Phenylalanine hydroxylase	Mental retardation	See tyrosine metabolism
	Tyrosinemia	-Tyrosine transaminase -p-hydroxyphenyl pyruvate oxidase.	Liver cirrhosis , Carcinoma and liver failure	
	Alkaptonuria	Homogentisate oxidase	Arthritis , dark urine	
	Albinism	Tyrosine hydroxylase	Defective synthesis of melanin pigments	
Tryptophan	Hartnup's disease	Amino acid transport system (intestine and renal tubules)	Pellagra skin rashes, psychiatric changes and mental retardation	See tryptophan metabolism
Cystine	Cystinuria	Renal tubular reabsorption of cystine ,lysine ,arginine and ornithine	Cystine renal calculi	See cysteine and cystine metabolism
	Cystinosis	Deposition of cystine in different tissues.	Amino aciuria and impaired renal functions	
Methionine	Homocystinuria	Cystathionine B-synthase	Mental retardation , thrombosis , osteoprosis	See fate of α-ketoacids
	Cystathioninuria	Cystathionase	No clinical symptoms	
Proline	Hyper-prolinaemia	Prolin oxidase	No clinical symptoms	See fate of α-ketoacids
Oxoproline	Oxoprolinuria (pyroglutamate aciduria)	Glutathione synthetase	Acidosis and neurologic disorders	See amino acids absorption
Ammonia, CO_2 , ATP	Hyper-ammoniemia I	Carbamoyl phosphate synthetase	Signs and symptoms of hyperammonemia	See sources and fate of ammonia
Ornithine	Hyper-ammonemia II	Ornithine transcarbomylase		
Citrulline	Citrullinemia	Arginosuccinate synthetase		
Argino-succinate	Arginosuccinate aciduria	Arginosuccinase		
Arginine	Argininaemia	Arginase		
Histidine	Histidinemia	Histidinase	Mental retardation	See fate of α-ketoacids
Valine, isoleucine and leucine	Maple syrup urine disease	α-ketoacid decarboxylase	Mental retardation and maple syrup odour of urine	See fate of α-ketoacids
Glycine	Glycinuria	Renal tubular reabsorption	Oxalate renal stones	See glycine metabolism
Glyoxylic acid	Iry hyperoxaluria	Failure to catabolize glyoxylate	Oxalate stones	See glycine metabolism

Chapter 2 — *Porphyrins, Heme and Hemoglobin metabolism*

I. *Introduction and definitions:*

A. **Porphin** is a cyclic compound formed by the linkage of 4 pyrrole rings (I, II, III and IV) through methenyl bridges (CH=).

B. **Porphyrins** are cyclic compounds in which there are side chains attached to the numbered positions of 4 pyrrole rings of porphin.

C. Porphyrins **differ from each other** in the type and arrangement of side chains attached to numbered positions.

D. The side chains which may be substituted in numbered positions are:

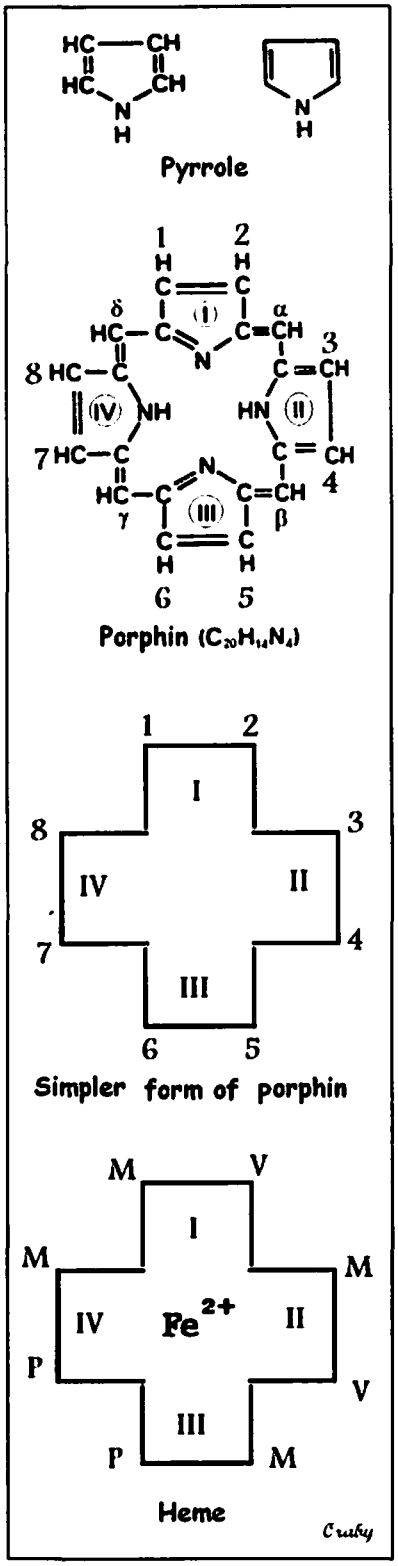
1. **A = Acetate =** $-CH_2-COOH$
2. **P = propionate=** $-CH_2-CH_2-COOH$
3. **M = Methyl =** $-CH_3$
4. **V = Vinyl =** $-CH=CH_2$

E. **Structure of heme:**

1. Heme is a complex of one of porphyrins called: **protoporphyrin III** and iron (in ferrous state, Fe^{++}) and Iron is held in the center of heme molecule by bonds to the 4 nitrogen atoms of pyrrole rings of protoporphyrin.

F. **Hemoproteins:**

1. These are a group of specialized proteins that contain heme as a prosthetic group. They are:
   a) Hemoglobin.
   b) Myoglobin.
   c) Respiratory cytochromes.
   d) Cytochrome P450.



e) Catalase and peroxidase.

f) Tryptophan oxygenase.

2. **The role of the heme group in each protein is:**

a) **In hemoglobin and myoglobin:** Heme acts as oxygen carrier.

b) **In cytochromes:** Heme acts as an electron carrier.

c) **In catalase and peroxidase:** Heme acts as a part of active site of the enzyme that catalyzes the breakdown of hydrogen peroxidase (H_2O_2). (For details, see iron metabolism).

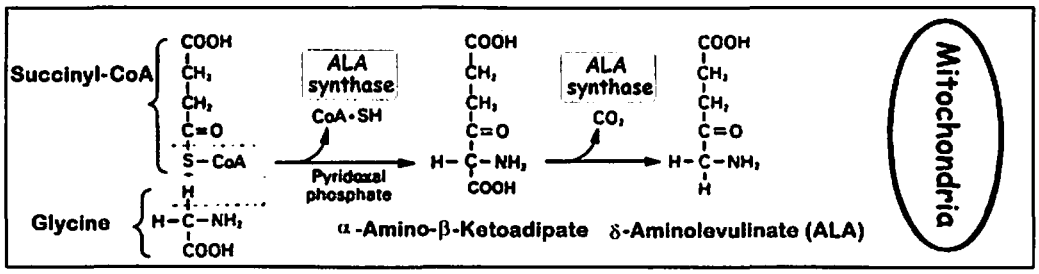
II. *Heme synthesis:*

A. Location:

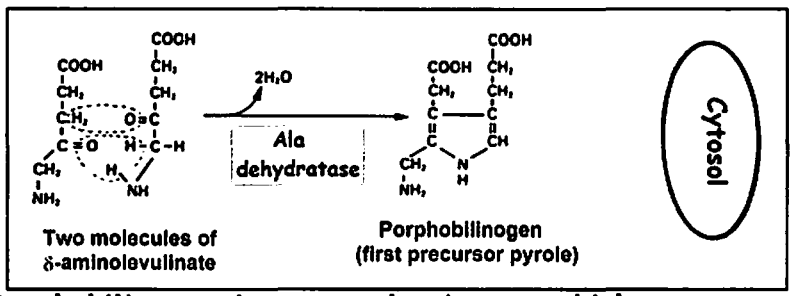
1. **Intracellular location:** Mitochondria, cytosol and then mitochondria again.
2. **Organ location: Liver and bone marrow.**

B. Steps:

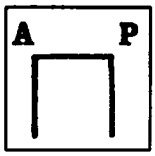
1. The 2 starting materials are **succinyl CoA** (derived from citric acid cycle in mitochondria) and **glycine.** The reactions occur in mitochondria and need aminolevulonic acid (ALA) synthase enzyme and pyridoxal phosphate as activator for glycine.

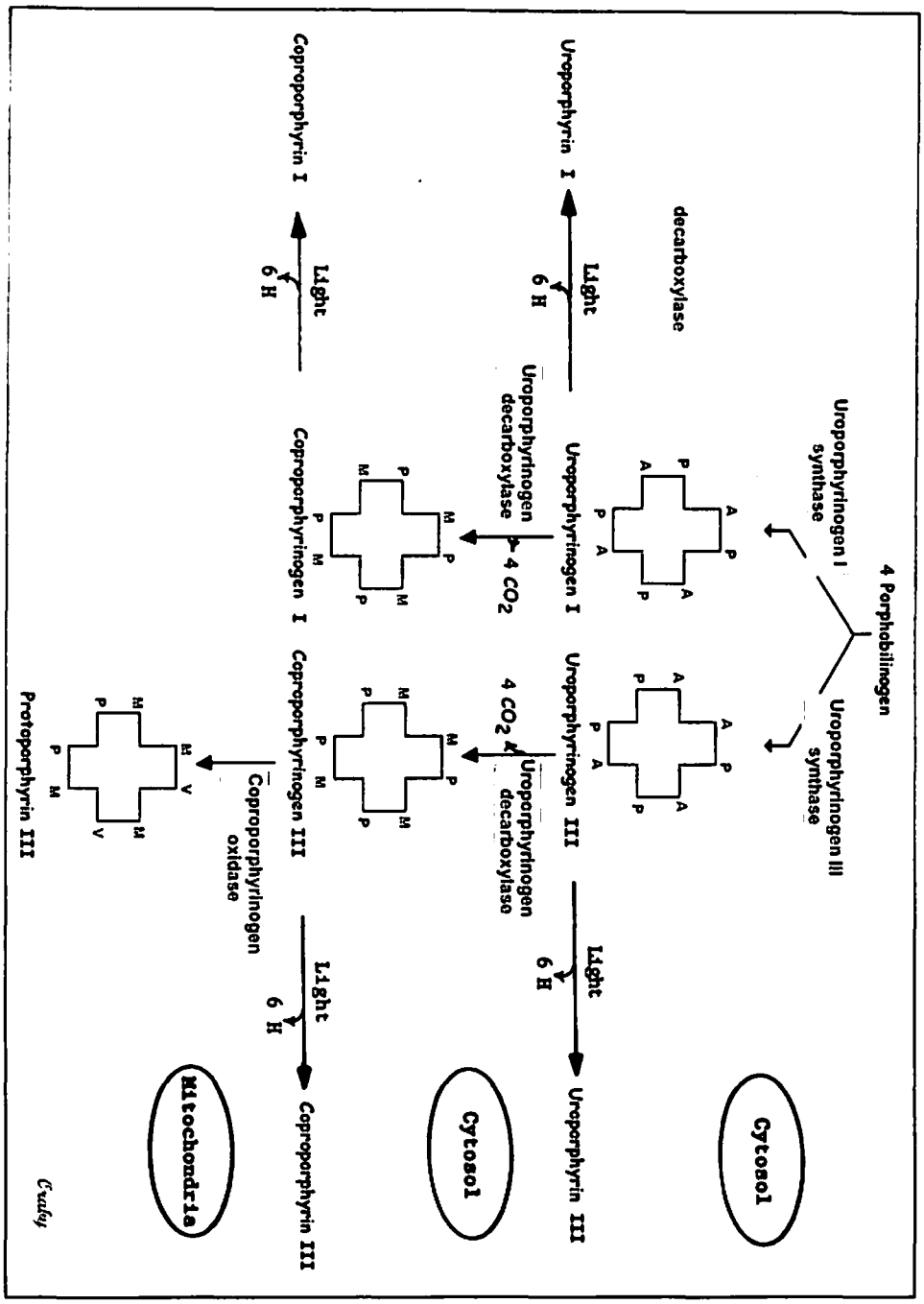


2. Then ALA passes from mitochondria to cytoplasm where 2 molecules are condensed to form porphobilinogen:

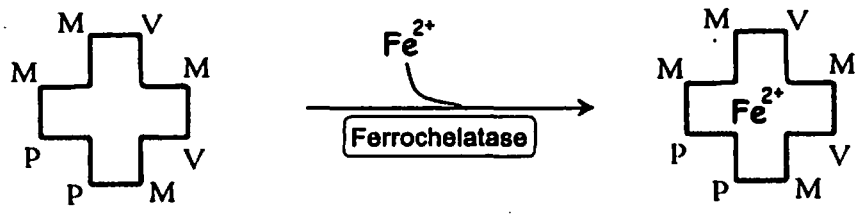


a) Porphobilinogen is a pyrrole ring to which a propionate (-CH2 -CH₂ -COOH), and acetate (-CH₂-COOH) groups are attached. Four porphobilinogen are condensed to give protoporphyrin as shown in the diagram as follows:





3. Then heme formation is completed by incorporation of iron (Fe^{++}) into protoporphyrin as follows:



Protoporphyrin III
(parent porphyrin of heme)

Heme
(prosthetic group of hemoglobin)

Comments on heme synthesis:

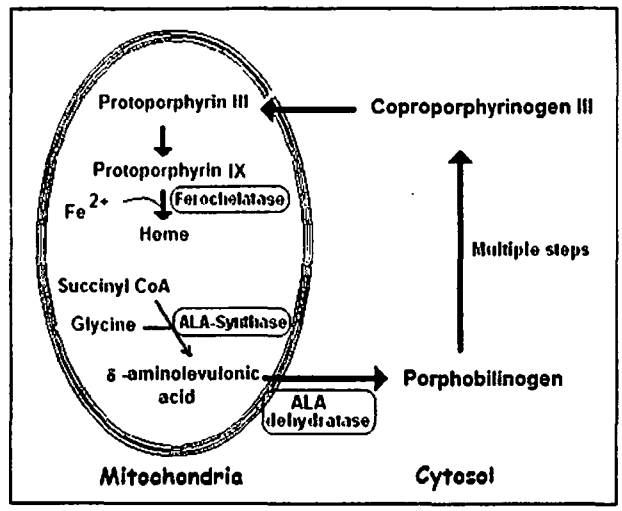
1. ***Uroporphyrinogen decarboxylase*** *present in cytosol removes carbon dioxide (CO_2) from 4 acetate groups (A), converting them into methyl groups (M).*

$$CH_2 - COOH \rightarrow -CH_3 + CO_2$$

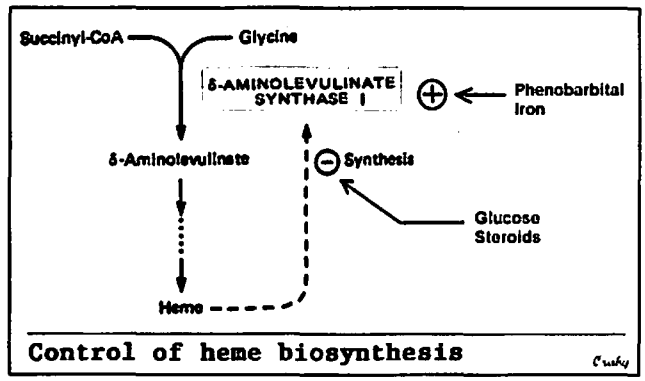
2. ***Coproporphyrinogen oxidase*** *presents in mitochondria catalyzes the decarboxylation, oxidation and dehydration of propionate groups (P) of ring I and II converting them into vinyl groups (V):*

$$-CH_2-CH_2-COOH \xrightarrow{-CO_2} -CH_2-CH_3 \xrightarrow{\pm O} CH_2-CH_2-OH \xrightarrow{-H_2O} -CH=CH_2$$

Propionate ***Ethyl*** ***Ethanol*** ***Vinyl (V)***



C. Regulation of heme synthesis:



This is done through hepatic *delta aminolevulinic acid synthase* enzyme ***(ALA synthase I)*** is the key enzyme in heme synthesis.

1. **It is inhibited by:**
   - *a)* Heme itself, by feed back inhibition.
   - *b)* Glucose and steroids.
2. **It is stimulated by:**
   - Certain drugs as phenobarbital and iron.

III. *Porphyrias:*

A. **Definition**: These are a group of diseases resulting from a deficiency of one of the enzymes needed for heme synthesis.

B. **Effects of porphyrias:** Porphyrias lead to disturbance in heme synthesis and cause:

1. **Anemia: due** to decrease production of heme.
2. **Abdominal pain and neuropsychatric symptoms** due to toxic effect of the accumulated porphyrin intermediates.
3. **Photosensitivity:** Some porphyrin derivatives when exposed to light react with molecular oxygen to form oxygen radicals, which cause skin damage.

C. Porphyrias are either **hereditary** or **acquired** (caused by environmental poisons as lead).

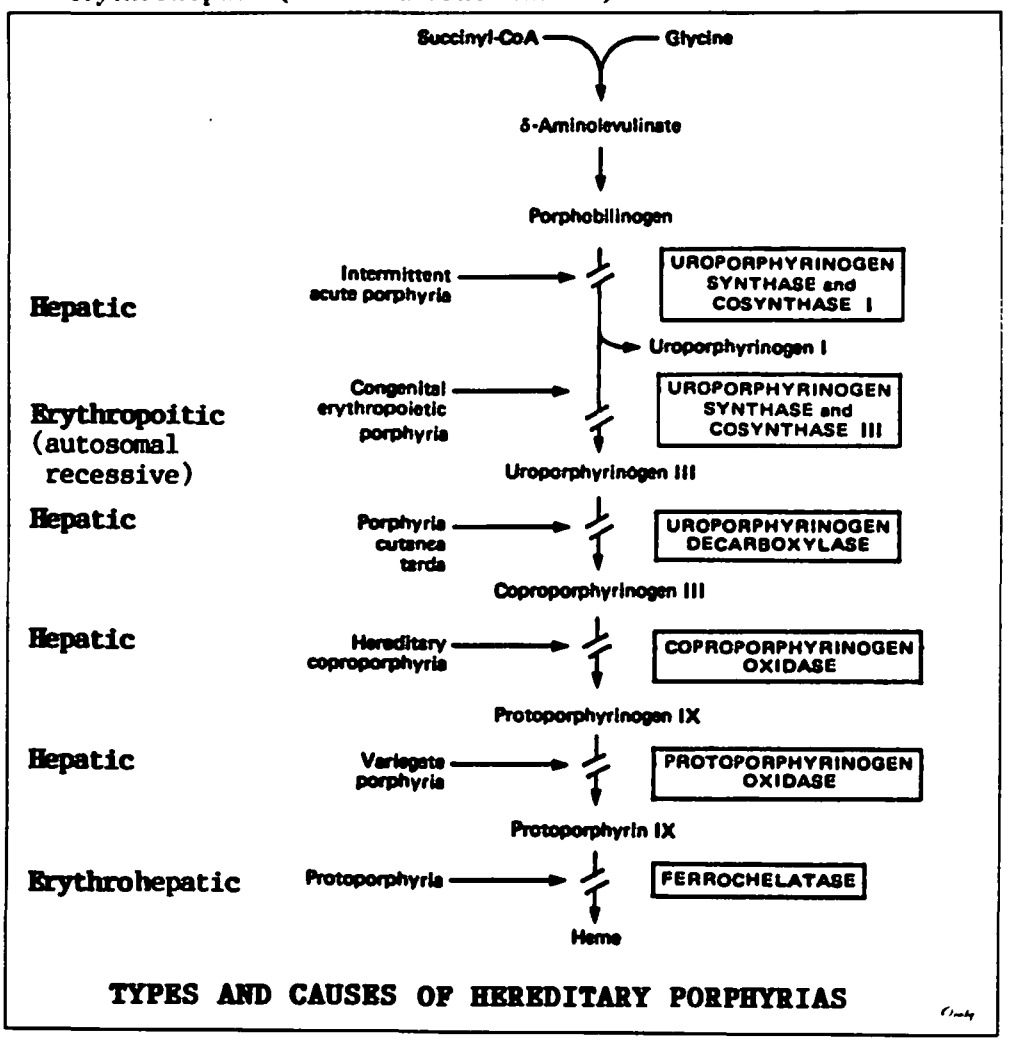
D. All hereditary porphyrias are **autosomal dominant** except congenital erythropoietic porphyria, which is **autosomal recessive**.

E. **Classification:**

1. The following table gives summary of the major findings of porphyrias:

Type and class	*Enzyme involved*	*Major symptoms*
Hepatic porphyrias:		
•Acute intermittent porphyria	Uroporphyrinogen I synthase	*Abdominal pain *Neuropsychatric
•Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	*Photosensitivity
•Hereditary coproporphyria	Coproporphyrinogen oxidase	*Abdominal pain *Neuropsychatric, *photosensitivity
•Variegate porphyria	Protoporphyrinogen oxidase	*Abdominal pain *Neuropsychatric, *photosensitivity
Erythropoietic porphyrias:		
•Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	*Photosensitivity
Erythrohepatic porphyrias:		
Protoporphyria	Ferrochelatase	*Photosensitivity

2. Liver and bone marrow are the organs where heme synthesis occurs.
3. According to the site of enzyme deficiency, porphyrias can be classified into hepatic (liver), erythropoietic (bone marrow) and erythrohepatic (liver and bone marrow).



TYPES AND CAUSES OF HEREDITARY PORPHYRIAS

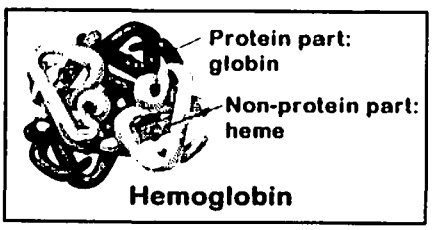
Hemoglobin metabolism

I. *Introduction:*

- Hemoglobin is found only in the red blood cells.
- Its main function is to transport oxygen from the lungs to the tissues and carbon dioxide (CO_2) from the tissues to the lungs.

II. *Structure of hemoglobin:*

A. Hemoglobin is conjugated protein, which consists of specialized protein called: **globin** that is tightly bound to 4 heme molecules.



B. Globin is a protein with four peptide chains joined together by non-covalent bonds (tetramer).

C. Types of hemoglobin: Several different kinds of hemoglobin molecules are normally found in human. They vary in the primary structure of the peptide chains of globin. These are HbA, HbA_2, HbF, HbA_1.

1. Hemoglobin A:

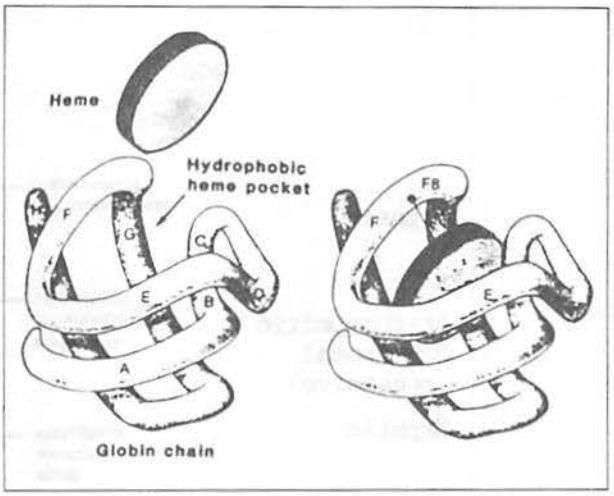
a) It is the major hemoglobin in adults (97 %).

b) Its globin comprises 4 polypeptide chains:

1) Two α-chains (141 amino acids).

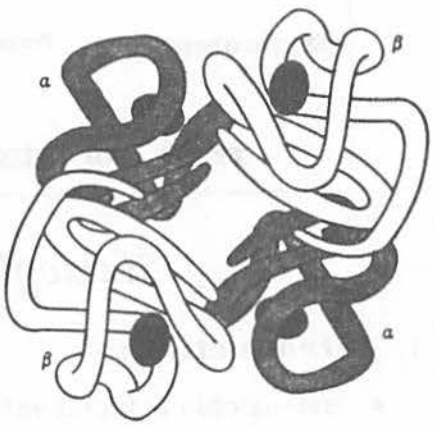
2) Two β-chains (146 amino acids).

3) This globin is abbreviated $\alpha_2\ \beta_2$.



c) Each polypeptide chain has a helical structure and folded into 8 stretches labeled A to H creating a pocket inside it for heme binding.

d) The interior portion of folded peptide chain is composed almost of nonpolar amino acids (hydrophobic). In contrast, polar amino acids are located almost on the surface of the molecule, where they can form hydrogen bonds with water (hydrophilic).



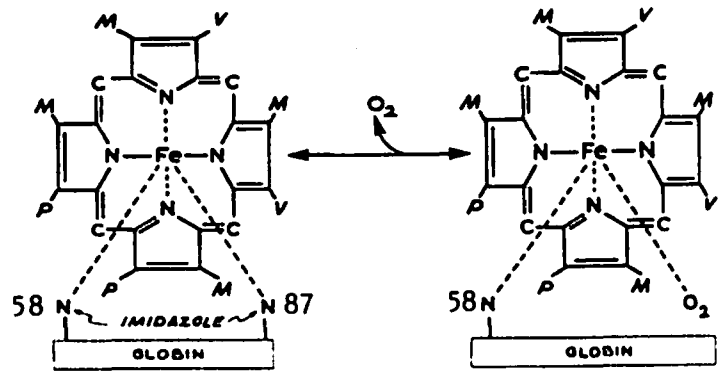
The quaternary structure of hemoglobin. Each of the 4 polypeptide chains of hemoglobin is represented by a continous band. The heme groups are represented by black disks.

e) The iron of each heme group is bound by coordination bonds to nitrogen atoms of imidazole rings of histidine amino acids:

1) Number 58 and 87 in α-chains.

2) Number 63 and 92 in β-chains.

f) When hemoglobin is oxygenated, the bonds between iron and histidine molecules, number 87 in α-chain and number 92 in β-chain are displaced by oxygen.



Reduced hemoglobin Oxyhemoglobin

Imidazole conjugation in hemoglobin.

2. Hemoglobin A_2:

a) It accounts about 2 % of adult human hemoglobin.

b) Its globin consists of 2 α-chains and 2 delta chains (δ): $\alpha_2\delta_2$.

3. Fetal hemoglobin (HbF):

a) This is hemoglobin present in the fetus during intrauterine fetal life.

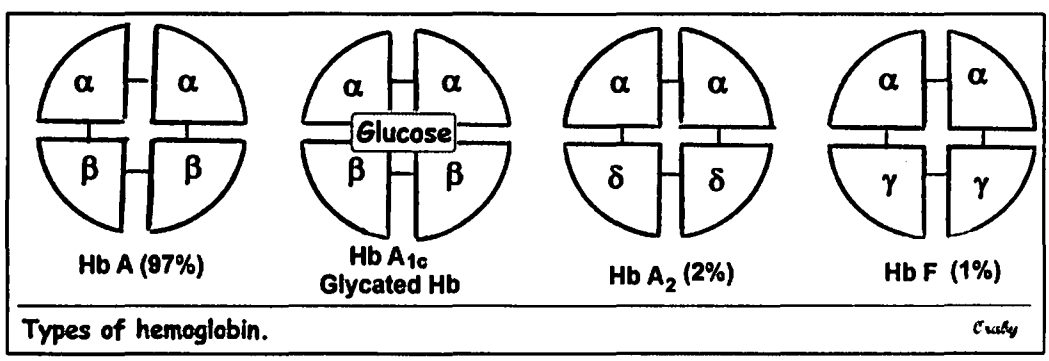
b) It consists of 2 α-chains and 2 gamma chains (γ): $(\alpha_2\gamma_2)$.

c) Hemoglobin F accounts about 1% of adult human hemoglobin.

4. Hemoglobin A_1 (Glycated hemoglobin):

a) Hemoglobin A reacts non enzymatically with glucose to form a derivative known as glycated hemoglobin or HbA_{1c}.

b) Normally the concentration of HbA_{1c} is very low (5-8 %) but in diabetes mellitus, where blood sugar levels are high, the concentration of HbA_{1c} may reach 12 % or more of the total hemoglobin concentration.

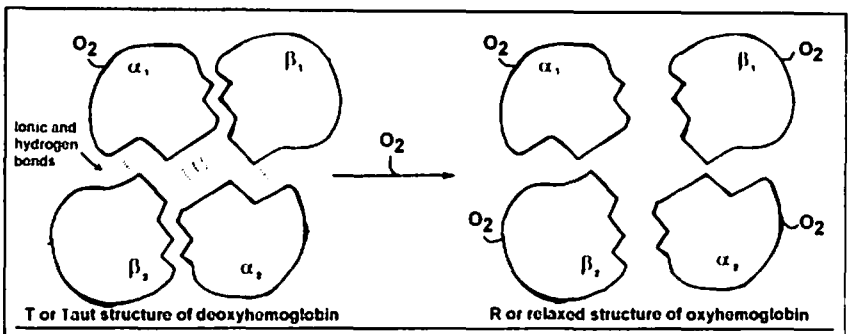


Types of hemoglobin.

III. *Factors affecting functions of hemoglobin:*

A. Cooperative binding of O_2:

1. **Definition**: This means that the binding of one oxygen molecule at one heme increases the oxygen affinity of the remaining heme groups in the same hemoglobin molecule.
2. There are 2 forms of hemoglobin T (tight), which is deoxyhemoglobin and R (Relaxed), which is oxyhemoglobin.
   a) In the T form, the 4 subunits are packed together by ionic bonds and hydrogen bonds.



The cooperative binding of O_2.

   b) The binding of O_2 to hemoglobin (T form) causes rupture of some of the ionic bonds and hydrogen bonds. This leads to a change of the T form to R or relaxed form, which has an affinity for the binding of oxygen two to three times greater than T form.

B. Bohr effects:

1. The decrease of pH and the increase of CO2 pressure at tissues ➔ lower the oxygen binding of hemoglobin and enhance the release of oxygen.
2. This change of oxygen binding is called **Bohr effect.**

C. Effect of 2,3 bisphosphoglycerate (2,3 BPG):

1. 2,3 BPG is synthesized from intermediates of glycolysis (see carbohydrate metabolism, part II).
2. 2,3 BPG is present in large amounts in RBCs.
3. 2,3 BPG decreases the oxygen affinity of hemoglobin by binding to deoxyhemoglobin but not to oxyhemoglobin. This leads to release of oxygen at tissues as follows:

$$HbO_2 + 2,3\ BPG \leftrightharpoons Hb\text{-}2,3\ BPG + O_2$$

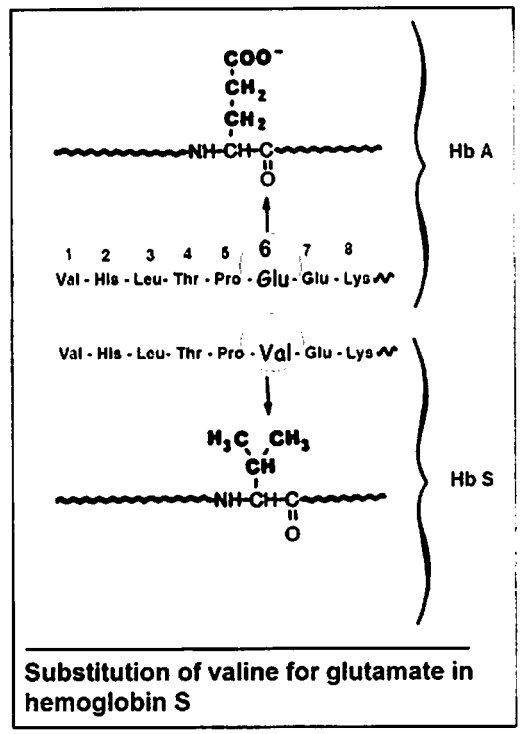
Oxyhemoglobin ***Deoxyhemoglobin***

IV. *Hemoglobinopathies:*

These are a group of diseases caused by either abnormal globin formation or synthesis of insufficient quantities of normal hemoglobin. Many disorders are present, but here, two of them will be discussed:

A. Sickle cell anemia:

1. The blood cells of these patients contain abnormal hemoglobin called **hemoglobin S (HbS).**
2. A molecule of HbS contains 2 normal α-chains and 2 mutant β-chains in which **glutamate** at position six has been replaced by **valine.**
3. Glutamate is polar while valine is nonpolar. This single error makes hemoglobin S less soluble especially in its deoxygenated form. **This will lead to:**
   a) The molecules of HbS aggregate to form fibers that deform red cells to give a **crescent or sickle shape.**
   b) Sickling of cells will block the flow of blood in small capillaries leading to **hypoxia, pain and death of cells** supplied by these capillaries.
   c) **Hemolysis** of RBCs.
4. **Two types of sickle cell anemia are present:**
   a) **Homozygous recessive disorder:** occurs in individuals who receive two mutant genes coding for synthesis of β-chains (one gene from father and the other from mother).
   b) **Heterozygous disorder: (sickle cell trait)** occurs in individuals having one normal gene and one sickle cell gene. Usually patients with sickle cell trait do not show clinical symptoms except if they are exposed to very low oxygen tension.

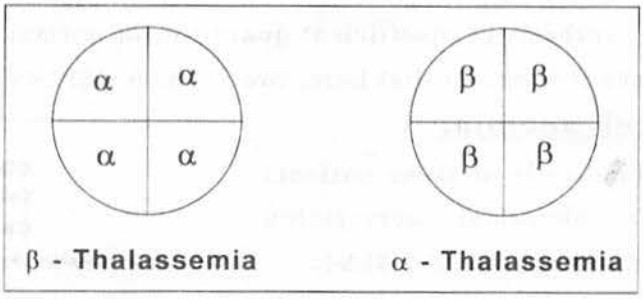


Substitution of valine for glutamate in hemoglobin S

B. Thalassemias:

1. Are anemias characterized by reduced synthesis of either alpha chain (α-thalassemia) or beta chain (β-thalassemia) of hemoglobin.
2. The causes are most often due to **gene deletions.**

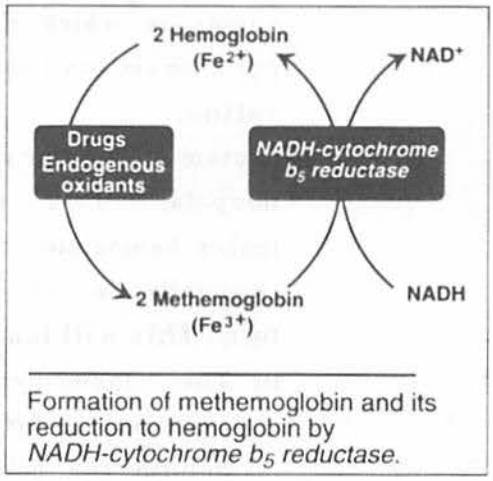
3. Thalassemia may be either **homozygous** with severe anemia or **heterozygous** (thalassemia trait) with no clinical symptoms.



V. *Abnormal derivatives of hemoglobin:*

A. Met-hemoglobin (Met-Hb):

1. It is oxidized hemoglobin in which the ferrous ions (Fe^{2+}) of hemoglobin is oxidized to the ferric state (Fe^{3+}).
2. Some drugs, endogenous oxidants, H_2O_2 and number of free radicals cause formation of Met-Hb.
3. **Met-Hb binds oxygen irreversibly and is unable to act as an oxygen carrier.**



Formation of methemoglobin and its reduction to hemoglobin by *NADH-cytochrome b_5 reductase.*

4. Met-Hb is reduced again into hemoglobin by NADH+H$^+$ cytochrome b_5 reductase enzyme. If Met-Hb is present in high concentration, it will lead to hypoxia and cyanosis.

B. Carboxy-hemoglobin (COHb):

1. It is hemoglobin combining with carbon monoxide(CO) .
2. Carbon monoxide combines at the same position in the Hb molecule as O_2, with affinity about 200 times greater than O_2.
3. Concentration of COHb above 40% usually results in unconsciousness, and may be fatal.

C. Sulf-hemoglobin (S-Hb):

1. It is hemoglobin combining with sulfur.
2. It results from exposure of hemoglobin to the toxic effects of certain drugs as sulfonamides.
3. S-Hb produces anoxia and cyanosis because it can not act as oxygen carrier.

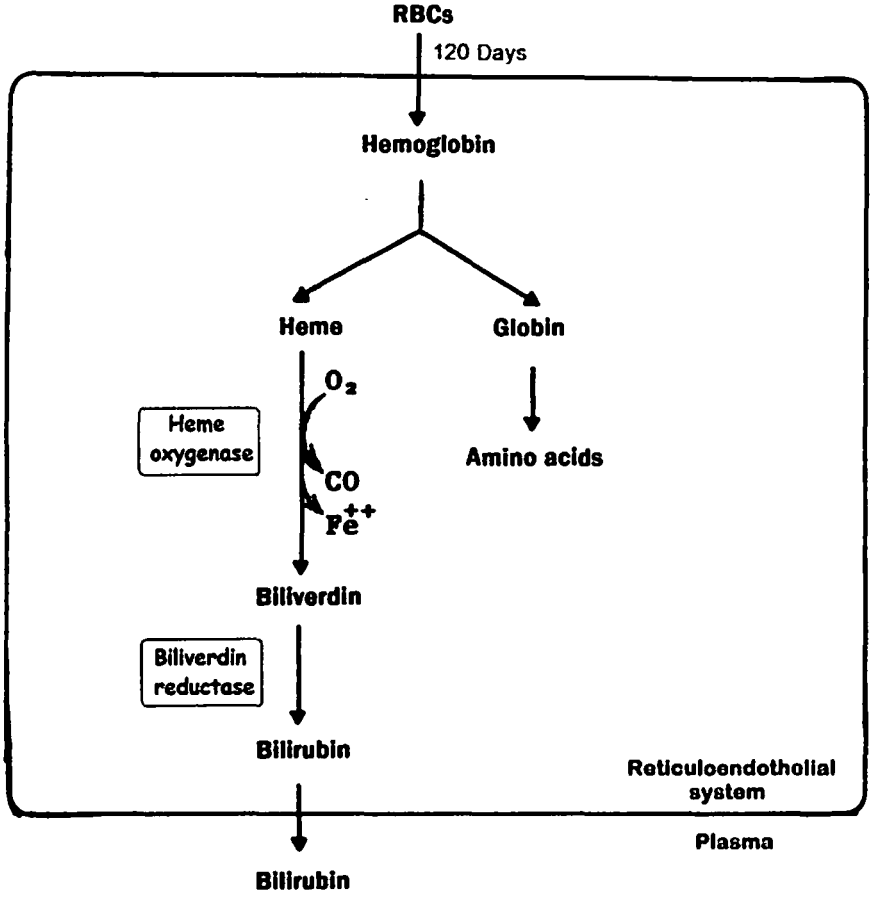
D. Hematin:

1. It is hemoglobin without iron (i.e. protoporphyrin combining with globin).
2. It may be formed following intravascular hemolysis.

VI. *Hemoglobin catabolism:*

A. Introduction:

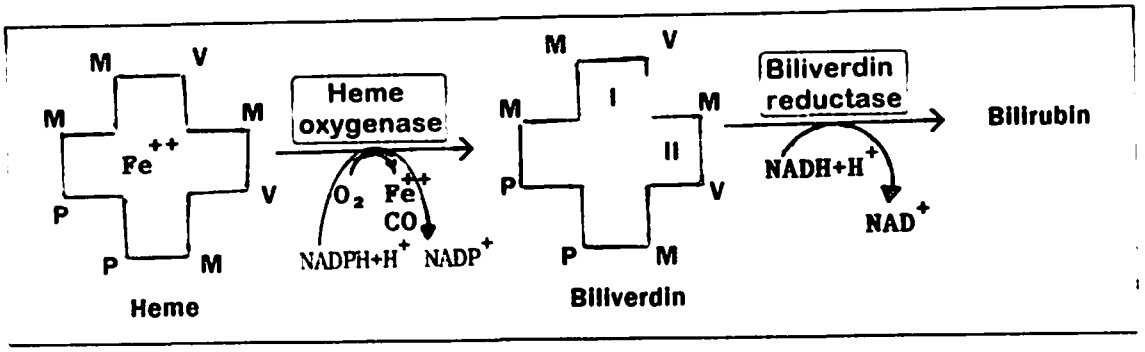
1. The average life span of the red blood cells is 120 days.
2. At the end of that time, they are removed from circulation by the cells of reticuloendothelial (RE) system mostly present in **liver, spleen and bone marrow**, where they are hemolysed (extravascular hemolysis) and hemoglobin comes out, giving globin and heme molecules.
   a) Globin molecule hydrolyzes into free amino acids.
   b) Heme gives iron and bilirubin as discussed below.



B. Formation of bilirubin:

1. The heme ring is catabolized by the microsomal heme oxygenase enzymes of the RE cells.
2. In this reaction (which needs, heme oxygenase enzyme, O_2 and NADPH), iron (Fe^{++}) is removed for re-use. The remaining of heme ring is cleaved between pyrrole rings number I and II to form biliverdin (green pigment) and carbon monoxide (CO).

3. Biliverdin is then reduced into bilirubin (golden yellow) in a reaction requires biliverdin reductase enzyme.

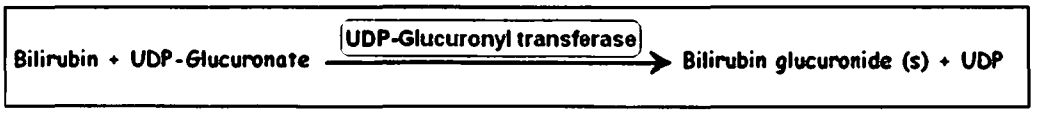


C. Transport of bilirubin in the plasma:

1. Bilirubin is nonpolar, and is insoluble in plasma. Therefore it binds by noncovalent bonds to plasma albumin. This form is called: unconjugated or indirect bilirubin.

D. Uptake of bilirubin by the liver:

1. Bilirubin dissociates from the carrier albumin molecule and enters hepatocytes.
2. Bilirubin is conjugated with one or two molecules of glucuronic acid (the acid form of glucose) to form bilirubin monoglucuronide and bilirubin diglucuronide. This reaction needs ***UDP-glucuronyltransferase*** enzyme:

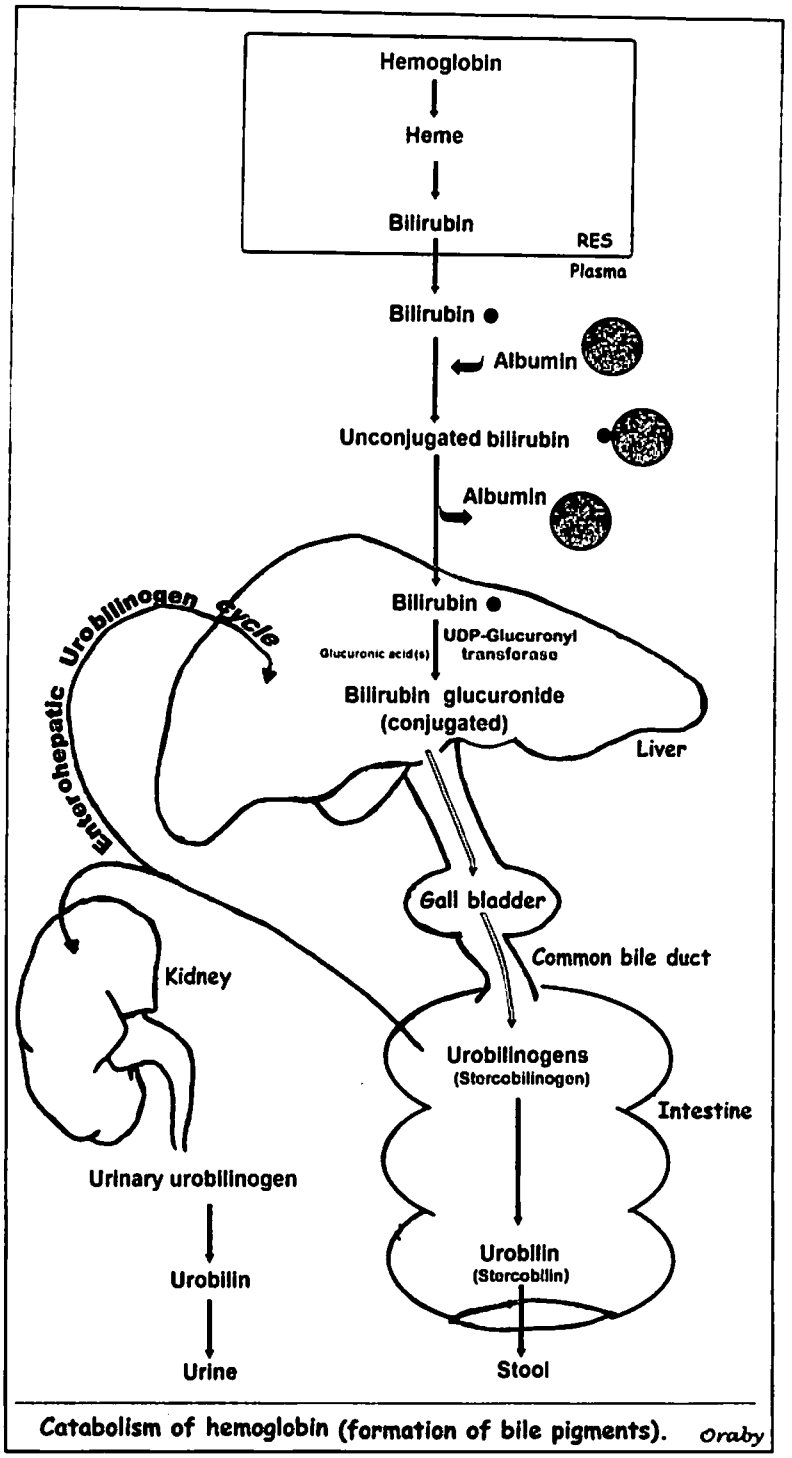


E. Secretion of bilirubin into bile:

Bilirubin diglucuronide is actively transported against concentration gradient into the bile canaliculi and then into the bile.

F. Formation of urobilin in the intestine:

1. Intestinal bacteria acts on bilirubin diglucuronide leading to:
   a) Removal of glucuronides (by β-glucuronidases enzymes).
   b) Reduction of bilirubin to colorless compounds called: **urobilinogens (=stercobilinogen).**
2. A small fraction of urobilinogens are reabsorbed from intestine to the liver again and re-excreted in the bile, forming the enterohepatic urobilinogens cycle.



Catabolism of hemoglobin (formation of bile pigments). Oraby

G. Excretion of urobilinogens in stool and urine:

1. Most of the colorless urobilinogens are oxidized to the colored **urobilin (stercobilin)**, which is excreted in the stool giving its brown color.
2. Part of urobilinogens are reabsorbed to the liver, then to the blood to be excreted by the kidney in urine and converted into urobilin.
3. Urobilin –together with urochrome- give the characteristic yellow color of urine.

VII. *Van den Bergh reaction:*

A. This is a reaction between bilirubin and Ehrlich diazo reagent giving a reddish purple compound.

B. Conjugated bilirubin reacts directly with the reagent. Thus it is called: **direct bilirubin.**

C. Unconjugated bilirubin does not react with the reagent directly except after addition of methyl alcohol. Thus it may be called: **indirect bilirubin.**

Differences between unconjugated bilirubin and conjugated bilirubin:

Unconjugated bilirubin	Conjugated bilirubin
1) Present normally in plasma.	1) Present normally in bile.
2) Attached non-covalently to albumin.	2) Conjugated to glucuronic acid.
3) Has high molecular weight and cannot be filtered through the kidney.	3) Has small molecular weight and if present in plasma can be filtered through the kidney.
4) Nonpolar, insoluble in plasma and can cross brain barrier in neonates causing brain damage.	4) Polar, soluble in plasma and can not cross brain barrier.
5) Gives indirect Van den Bergh reaction.	5) Gives direct Van den Bergh reaction.

Hyperbilirubinemia And Jaundice

I. *Introduction:*

A. **Plasma bilirubin concentration:** = 1.2 mg/dl (1 mg =17.1 umol/L).

B. **Hyperbilirubinemia:**

1. It is increased plasma bilirubin above normal level (above 1.2 mg/dl).
2. It may be due to increase conjugated and/or unconjugated bilirubin(s).

C. **Jaundice (icterus):**

1. It is a clinical condition characterized by **yellow** discoloration of skin, sclera and mucus membrane.
2. It is due to increase plasma bilirubin above 3 mg/dl. At this level, bilirubin diffuses into tissues giving yellow color.

> ***Note:***
> *There is a difference between the term **hyperbilirubinemia**, which is a **laboratory** term, and **jaundice**, which is a **clinical** term. Also hyperbilirubinemia may be present (1.2-3 mg/dl) without appearance of jaundice.*

II. *Types Of Hyperbilirubinemia:*

- Neonatal hyperbilirubinemia.
- Pathological hyperbilirubinemia.
- Congental hyperbilirubinemia.

Neonatal "Physiologic" Hyperbilirubinemia (Jaundice)

A. **Definition:** This is a transient condition that occurs in some newborn infants especially if they are premature.

B. **Causes:**

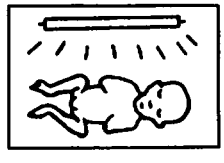
1. At birth, liver contains very little UDP-glucuronyl-transferase enzyme, which is important for conjugation of bilirubin.
2. At birth, there is an accelerated hemolysis of RBCs.

C. **Effects:**

1. **This leads to** increased unconjugated bilirubin and jaundice which lasts 2-3 days in full term infants and about 6 days in premature infants.
2. **Kernicterus:** If unconjugated bilirubin exceeds the concentration, which can be tightly bound to plasma albumin (20-25 mg/dl), free bilirubin can pass blood brain barrier, causing damage to the brain centers of infants. This is called Kernicterus. It may cause **mental retardation.**
3. Kernicterus develops because the excess bilirubin is soluble in the lipid of the basal ganglia of the brain.

D. **Treatment :**

Neonatal jaundice is treated by phenobarbital and exposure of jaundiced baby to ultraviolet light (photo- therapy) as bilirubin is broken down in light.



Pathological Hyperbilirubinemia (Jaundice)

It is either: prehepatic, hepatic or posthepatic.

Causes of jaundice:
- Pre-hepatic
- Hepatic
- Post-hepatic

A. **Prehepatic hyperbilirubinemia:**
(hemolytic jaundice):

1. Hyperbilirubinemia is characterized by increased **unconjugated** bilirubin. It occurs in all types of hemolytic anemia i.e. excessive destruction of RBCs inside blood vessels (intravascular hemolysis).
2. It is due to increase in the amount of plasma unconjugated bilirubin more than the capacity of the liver.
3. **Biochemical changes:**
   a) Increased production of bilirubin leads to increased production of urobilinogen, which appears in urine in large amounts.

b) No bilirubin appears in urine. (So that the combination of increased urobilinogen and absence of bilirubin in urine is suggestive of hemolytic jaundice).

B. Hepatic hyperbilirubinemia: (hepatocellular jaundice):

1. It is due to liver cells damage by cirrhosis, infective hepatitis or toxins, usually there is an associated obstruction of some biliary canaliculi.
2. Hyperbilirubinemia is characterized by increased both unconjugated and conjugated bilirubin.
3. **Biochemical changes:**
   a) Urobilinogen appears in normal trace amounts.
   b) Bilirubin also appears in urine.

C. Post-hepatic hyperbilirubinemia: (cholestatic or obstructive jaundice):

1. Cholestasis (stoppage of bile flow) may be due to mechanical obstruction of biliary tree by gallstone in common bile duct, carcinoma of the biliary tree or cancer head of pancreas (which exerts pressure on biliary tract).
2. Hyperbilirubinemia is mostly of conjugated type.
3. **Biochemical changes :**
   a) Urobilinogen is absent in urine.
   b) Urobilinogen is absent in stool giving clay color stool.
   c) Bilirubin appears in urine.
4. In obstructive jaundice, bilirubin together with bile salts returns to blood. **Increased bile salts in blood leads to:**
   a) **Itching** because bile salts are irritant to sensory nerves.
   b) **Bradycardia** because bile salts are toxic to cardiac muscles.

Laboratory results in normal subjects and patients with 3 different causes of jaundice:

Condition	*Serum bilirubin mg / dl*	*Urine uro-bilinogens mg / 24 h*	*Urine bilirubin*	*Fecal uro-bilinogens mg / 24 h*
Normal	Indirect: 0.2-1.2 Direct: 0.0-0.2 Total: 0.2-1.2	0-3	Absent	30-300
Hemolytic anemia	Elevation of indirect	Increased	Absent	Increased
Hepatitis	Elevation of direct and indirect	Normal	Present	Decreased
Obstructive jaundice	Elevation of direct	Absent	Present	Absent

Congenital hyperbilirubinemia:

A. **Gilbert's disease :**

1. It is due to defect in the **uptake** of bilirubin by the liver cells (due to a mild **deficiency of UDP-glucuronyltransferase).**
2. It is asymptomatic **unconjugated hyperbilirubinemia.**
3. Bilirubin concentration is usually less than 3 mg / dl.

B. **Crigler - Najjar syndrome:**

1. It is due to defect on the **conjugation** of bilirubin (due to **absence of UDP-glucuronyltransferase).**
2. It is a rare autosomal recessive disorder.
3. **There are two types of Crigler-Najjar syndrome:**

a) **Type I:**

1) It is more **severe** unconjugated Hyperbilirubinemia. Bilirubin concentration is usually **exceeds 20** mg / dl.
2) It occurs in neonates, leading to **kernictrus** and often to early **death** in the first 15 months of life.
3) The patient does not respond to barbiturate treatment.

b) **Type II:**

1) It is **milder** form (bilirubin usually **below 20** mg/dl) and not fatal.
2) The patient responds to high doses of barbiturate.

C. **Dubin - Johnson syndrome:**

1. **It is a conjugated hyperbilirubinemia, which occurs during adult life.**
2. **It is due to defect in the hepatic secretion of conjugated bilirubin into the bile.**
3. **It is not fatal disease.**

III. *Delta bilirubin:*

A. This type of bilirubin appears in plasma of patients with chronic obstructive jaundice.

B. In these patients some conjugated bilirubins react covalently with albumin in liver cells forming bilirubin-albumin complex, called δ-bilirubin.

C. It gives direct Van den Bergh reaction i.e. it is direct bilirubin. **The covalent binding and open configuration of bilirubin molecule is the cause of this direct reaction.**

D. This fraction has longer half-life in plasma than conjugated bilirubin. This explains the persistence of yellow tinge of the sclera some days after conjugated bilirubin level has returned to normal.

Chapter 3 *Body Fluids*

Introduction:

Body contains many fluids, which differ in composition to meet their functions. The most important body fluids are blood, urine, milk, semen, cerebrospinal fluid, aqueous humor, sweat, tears, lymph, amniotic fluid, synovial fluid, pleural, pericardial and peritoneal fluids.

Blood

I. *Definitions:*

A. Blood is a liquid consisted of a yellowish fluid called **plasma** in which **red cells, white cells** and **platelets** are suspended.

B. Once the blood has clotted (coagulated), the remaining liquid is called **serum.** Thus, serum is plasma without clotting factors.

II. *Functions of blood:*

1. **Respiration:** transport of oxygen from the lungs to the tissues and of CO_2 from the tissues to the lungs.
2. **Nutrition:** transport of absorbed food materials.
3. **Excretion:** transport of metabolic waste to the kidneys, lungs, skin, and intestines for removal.
4. Maintenance of normal **acid - base balance** in the body.
5. Regulation of **water balance** through the effects of blood on the exchange of water between the circulating fluid and the tissue fluid.
6. Regulation of **body temperature** by the distribution of body heat.
7. **Defense** against infection by the white blood cells (lymphocytes) and circulating antibodies.
8. Transport of **hormones** and regulation of metabolism.
9. Transport of **metabolites.**
10. **Coagulation.**

III. *Composition of plasma:* It consists of:

A. **Water:** about **90 %.**

B. **Solids:** about **10 %.** They include:

1. **Organic matters:** proteins, lipids (plasma lipoproteins), carbohydrate (glucose and other blood sugars), non-protein nitrogenous compounds (amino acids, urea, uric acid, creatinine, etc), hormones, enzymes, ketone bodies and other organic compounds.
2. **Inorganic matters:** include plasma electrolytes, Na^+, K^+, Ca^{++}, Cl^- and carbon dioxide (CO_2).

IV. *Plasma proteins:*

The concentration of total proteins in human plasma is approximately **6–9 g / dl** and they comprise the major part of solids in plasma. There are many protein types, some of them are **simple proteins** and others are **glycoproteins.**

A. Types of plasma proteins:

1. **Pre-albumin: 25 mg/dl:**
   It is responsible for **transport** of T_3, T_4 and retinal.
2. **Albumin: 4.5 g/dl:**
   a) It is the most abundant plasma protein of M.W. 68,000.
   b) **Its functions** are maintenance of **plasma osmotic pressure** and it acts as **transport carrier** for calcium, bilirubin, fatty acids and aldosterone in blood.
3. **Globulins: 2.7 g/dl:** Their M.W. ranges 90,000-1000,000. They are further subclassified into:
   a) **α-1-Globulins: prothrombin** (for blood clotting), **Retinol binding globulin** (for retinol transport), **transcortin** (for cortisol transport), **vitamin D-binding globulin** (for vitamin D transport), α_1-antitrypsin, α_1-acid glycoprotein and α_1-fetoprotein.
   b) **α-2-Globulins:** They include: **ceruloplasmin** (for copper transport), **haptoglobin** (for plasma hemoglobin binding), **α_2-macroglobulin** (it has antiprotease and transport functions) and **thyroxin-binding globulin** (for T_3 and T_4 transport).
   c) **β-Globulins:** They include: **plasminogen** (for fibrinolysis), **transferrin** (for iron transport), **C-reactive proteins, β_2-microglobulin.**

d) **Gamma globulins:** These are antibodies which are: **IgG, IgA, IgM , IgE** and **IgD** (see immunochemistry).

B. Functions of plasma proteins :

1. Maintenance of plasma **osmotic pressure,** mainly by albumin. Hypoproteinemia leads to edema.
2. **Transport functions:** many plasma proteins act as a carrier proteins of: lipids, hormones (e.g. thyroxin, cortisol), metals (e.g. calcium, copper and iron) and excretory products (e.g. bilirubin).
3. **Defense reactions** by immunoglobulins.
4. **Coagulation and fibrinolysis.**
5. **Buffering** of H^+ ions.
6. **Special functions,** including protease inhibitors e.g. α_1-antitrypsin and, α_2-macroglobulin.

C. Organs for synthesis of plasma proteins:

1. **Liver:** All plasma proteins - except gamma globulins- are synthesized in the **liver.**
2. **Lymphocytes:** Gamma globulins (antibodies) are synthesized by plasma cells in **lymphoreticular system.**

D. Albumin / globulin ratio (A/G ratio):

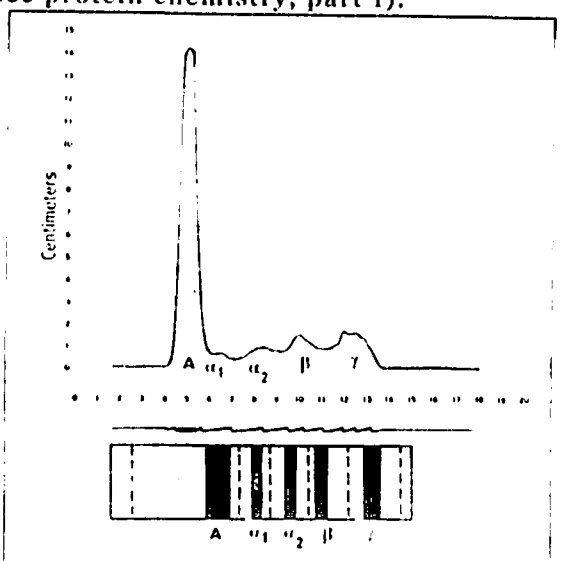
It is about **1.6/1.** This ratio is inverted in:

1. **liver diseases** (due to decreased albumin synthesis)
2. **Kidney diseases** (due to loss of more albumin than globulins as albumin has smaller molecular weight).

E. Methods of measurement and separation of plasma proteins:

1. **Direct chemical measurement** e.g. Biuret method for detecting the presence of peptide bonds. This method measures the total concentration of proteins.
2. **Measurement of biological activity** e.g. enzymatic activity, coagulation properties.
3. **Immunological methods** using antigen-antibody reactions.
4. **Physical measurements** e.g. **nephelometry,** where scattered light by protein particles is measured.

5. **Measurement after separation** by techniques such as electrophoresis, isoelectric focusing, chromatography, ultracentrifugation, precipitation (by salts or alcohol) and dialysis (see protein chemistry, part I).



Cellulose acetate electrophoresis pattern of normal human serum. A = Albumin, α_1 & α_2 = α_1 and α_2 globulins, β = β globulin and γ = γ globulins

V. *Plasma enzymes:*

A. Introduction:

1. Enzymes present in plasma are either functional or nonfunctional.
   a) The functional enzymes are those, which perform a physiologic function in, blood e.g. lipoprotein lipase and enzymes of fibrinolysis and coagulation.
   b) The non-functional plasma enzymes are those, which perform no known physiologic function e.g. lipase and amylase.
2. If a disease causes cell damage of an organ , which produces non-functional enzymes, the plasma levels of its enzymes are elevated and can be used in clinical diagnosis.

B. Types of enzymes of clinical importance:

1. **Transaminases (ALT and AST):**
   These enzymes are present in most tissues, but especially in cardiac muscle and liver.
   a) **ALT activity:** is widely used as a test for diagnosis of **hepatocellular damage e.g.** acute viral hepatitis.
   b) **AST activity:**
      1) It is also used for diagnosis of hepatocellular damage.
      2) It is increased in **myocardial infarction.** It gets its maximum level after 2 days of attack.

2. **Alkaline phosphatase:**
   a) It shows its maximum activity in the range of pH 9.0-10.5.
   b) Liver, bone, placenta and intestine are important sources of plasma alkaline phosphatase:
      1) **Physiological increase:** of alkaline phosphatase occurs in **growing children** (bone) and in **pregnancy** (placenta).
      2) **Pathological increase:** occurs in **rickets** and **hyperparathyroidism** (bone) and in **obstructive jaundice** (liver).

3. **Acid phosphatase:**
   It shows its maximum activity in the range of pH 4-5. The prostate contains high concentrations of acid phosphatase, and its measurement is used mainly for the diagnosis of **porstatic carcinoma.**

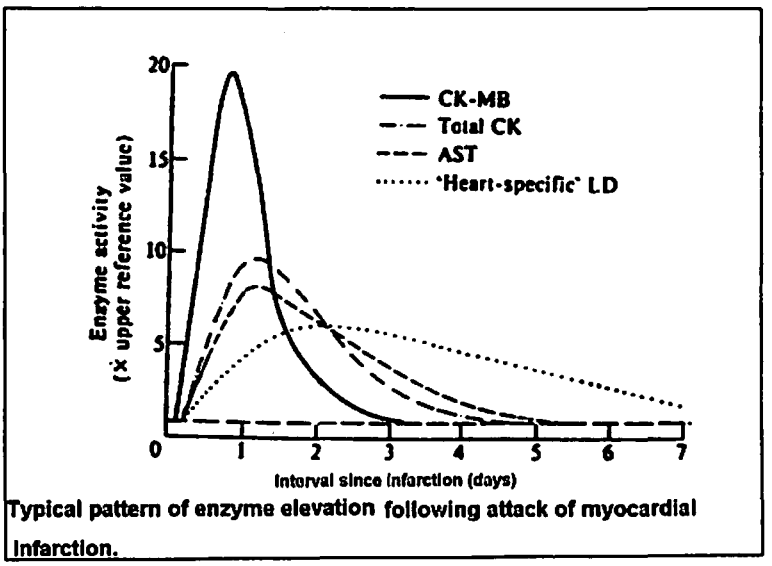
4. **Lactate dehydrogenase (LD):**
   It is present in most tissues especially liver, heart and muscles. Its activity is increased in hepatitis, myocardial infarction and muscle diseases.
   - In myocardial infarction, LD gets its maximum level after 5 days and returns to normal after 5-7 days of attack.

5. **Amylase:**
   It is produced by pancreas and parotid glands. Its activity increases in acute pancreatitis and parotitis.

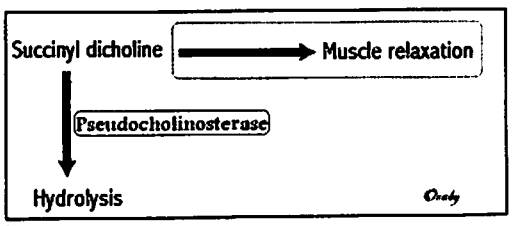
6. **Lipase:**
   It is produced by pancreas. Its activity increases in acute pancreatitis and pancreatic carcinoma.

7. **Creatine kinase (CK):**
   Also known as creatine phosphokinase (CPK). It is increased in myocardial infarction and in myopathies, in myocardial infarction; it gets its maximum level after 24 hours, and returns to normal level within 2-3 days.

> **Note:**
> **Enzymes for diagnosis of myocardial infarction:**
> 1- First 24 hours: CPK
> 2- 2-3 Days: AST
> 3- 5-7 Days: LDL



Typical pattern of enzyme elevation following attack of myocardial infarction.



8. **Acetylcholinesterase:**
   a) An enzyme of postsynaptic membrane that degrades the neurotransmitter acetylcholine.
   b) There are 2 types of the enzyme:
      1) Plasma acetylcholinesterase: known as pseudocholinosterase.
      2) Tissue acetylcholinoesterase: known as true cholinoesterase.
   c) **Succinyl choline apnea:** Some patients during anesthesia and after administration of **succinyl dicholine** as muscle relaxant develop prolonged apnea, often lasting for several hours. The plasma of these patients is usually deficient in pseudocholinesterase enzyme essential for hydrolysis of succinyl dicholine.
9. **Gamma-glutamyl transferase (GGT):**
   Also known as gamma glutamyl transpeptidase. It is found in a number of tissues especially kidney and liver. Its activity increases in cholestasis (i.e. impairment of bile flow) and in 70-80% of chronic alcoholics.

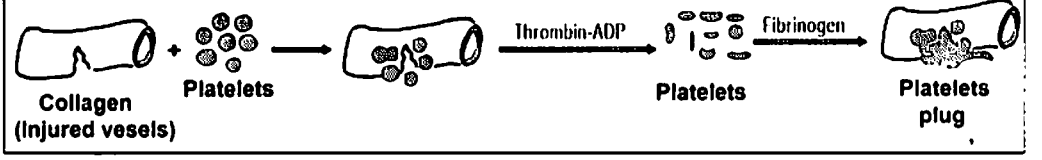
VI. *Hemostasis and blood coagulation:*

A. Hemostasis is the cessation of bleeding that follows injury of blood vessels.

B. Mechanisms responsible for cessation of bleeding: When blood vessel is injured, bleeding stops by the following mechanisms:

1. **Constriction** of the injured vessel to diminish blood flow.

2. **Formation of a loose and temporary platelet plug** (white thrombus):
   At the site of injury: **collagens** of blood vessels will be exposed → Platelets bind to the collagen (and **activated by thrombin** or **ADP**) → Platelets change shape (and **in presence of fibrinogen**) → Platelets aggregation → Formation of platelets plug → Stop bleeding. This mechanism is measured by **bleeding time.**



3. **Formation of fibrin mesh or clot (coagulation)**; that contains the platelet plug (white thrombus) and / or red cells (red thrombus) forming a more stable thrombus.
4. Partial or complete **dissolution of the clot by plasmin (fibrinolysis).**

In normal hemostasis, there is a dynamic steady state in which thrombi are constantly being formed (by coagulation) and dissolved (by fibrinolysis).

C. <u>Mechanism of blood coagulation</u>: Two pathways lead to fibrin clot formation, intrinsic and extrinsic pathways:

- **Intrinsic pathway**: It occurs in areas without a tissue injury due to either **restricted blood flow** or in response to **abnormal vessel wall.** Theoretically, this pathway may be divided into 3 stages :

1. **Generation of active factor X (Xa):**
   a) When blood vessel is disrupted, its **collagen** will be exposed. Collagen acts as a **negatively charged activating surface** which activates prekallikrein into **kallikerein.** Kallikerein activates factor XII into active factor XII **(XIIa).**
   The active factor XIIa attacks:
   1) **Prekallikerein** to generate more **kallikerein,** setting up a reciprocal activation. High molecular kininogen **(HMK)** participates as non enzymatic accelerator of this reaction.
   2) **High molecular weight kininogen** to generate **bradykinin.**
   3) **Factor XI,** in the presence of HMK as a cofactor, activating it into **active factor XIa.**

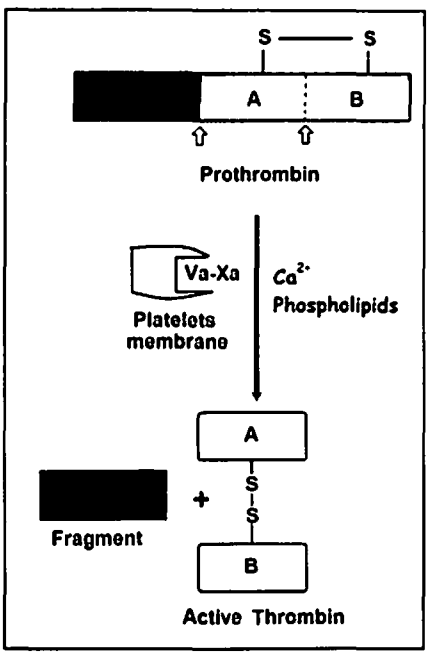
b) Factor XIa in the presence of Ca^{2+} ions activates factor **IX** to serine protease active factor IX (**IXa**),

c) Active factor IXa activates **factor X** into serine protease active factor X (**Xa**). This activation is accelerated about 500 folds in the presence of **phospholipids , Ca^{2+} and factor VIIIa**, (phospholipids, Ca^{2+}, Factor VIIIa and IXa are called **tenase complex).**

1) This reaction occurs on the **platelet surface.**
2) Phospholipids are derived from activated platelets.
3) Factor VIII is activated into VIIIa by a minute amount of thrombin. It acts as a receptor for factors IXa and X.
4) Formation of factor Xa occurs at the site where the intrinsic and extrinsic pathways start a final common pathway of blood coagulation.

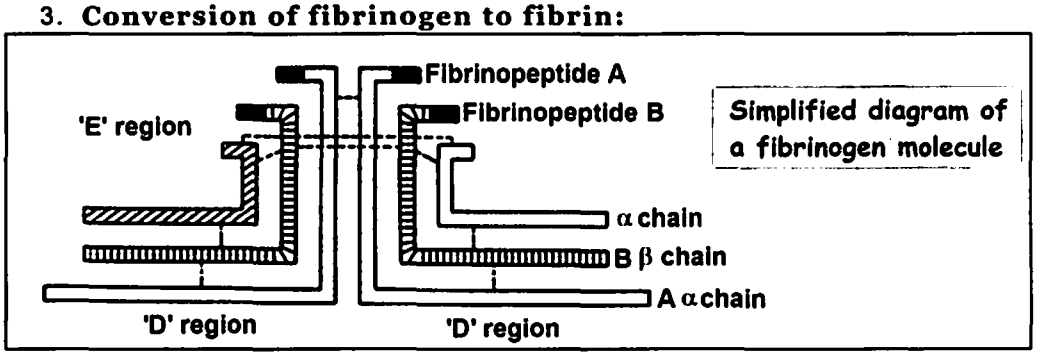
2. **Conversion of prothrombin into thrombin:**

a) **In the final common pathway,** factor Xa activates prothrombin (factor II) to thrombin (factor IIa). The activation of prothrombin occurs on the surface of **activated platelets** and requires phospholipids (from platelets), Ca^{2+}, factors Xa and Va.

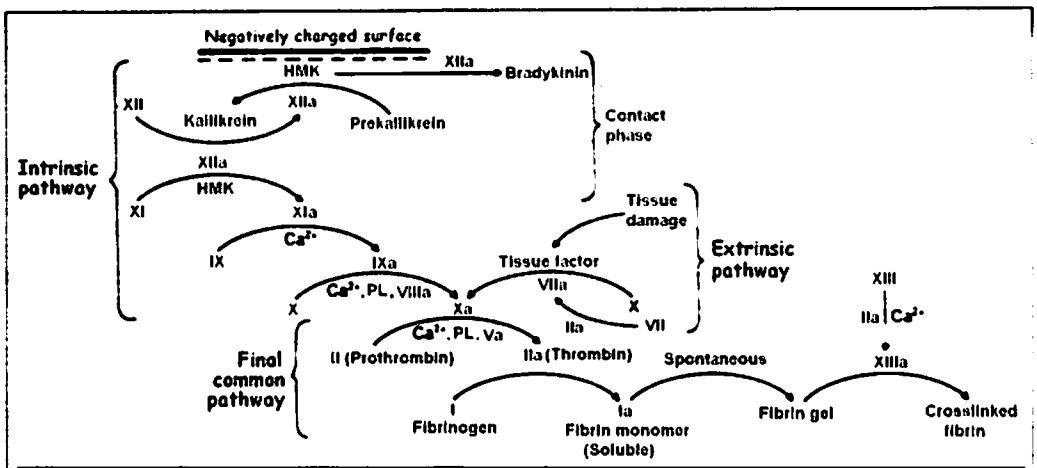


b) **Factor V is activated** by a minute amount of thrombin into factor Va, which then binds with specific receptors on the platelet membrane, and form complex with factor Xa and prothrombin. This complex in presence of Ca^{2+} and phospholipids activates prothrombin to thrombin.

3. **Conversion of fibrinogen to fibrin:**



a) Fibrinogen (factor I) is a soluble plasma glycoprotein that consists of 3 non identical pairs of polypeptide chains covalently linked by disulfide bonds.



Intrinsic and extrinsic pathways of blood coagulation.

b) Thrombin, a serine protease causes conversion of fibrinogen to fibrin by releasing of **fibrinopeptide portions** (the black areas) of fibrinogen, converting it into **fibrin monomer.** Fibrin monomers aggregate spontaneously to form **fibrin gel,** which in the presence of **active factor XIIIa,** thrombin (IIa) and Ca^{2+}, is converted to **insoluble fibrin clot.** This clot traps platelets, red cells and other components to form white or red thrombi. Factor XIII is activated into active factor XIII (XIIIa) by thrombin.

- **Extrinsic pathway**: It occurs at the site of tissue injury with the release of tissue factor that acts as a cofactor for active factor VII (VIIa).

1. Factor VII is activated into active factor VII (VIIa) by a minute amount of thrombin. Active factor VII acts as serine protease and together with tissue factor, they activate factor X into active factor X (Xa).
2. Factor Xa then proceeds in the final common pathway as in the intrinsic pathway.

D. Important notes on blood coagulation :

1. **Coagulation factors involve** factor I, II, III, IV, V, VII, VIII, IX, X, XI, XII, XIII as well as prekalikerein, and high M.W. Kininogen. The names and numbers of factors are listed in the following table:

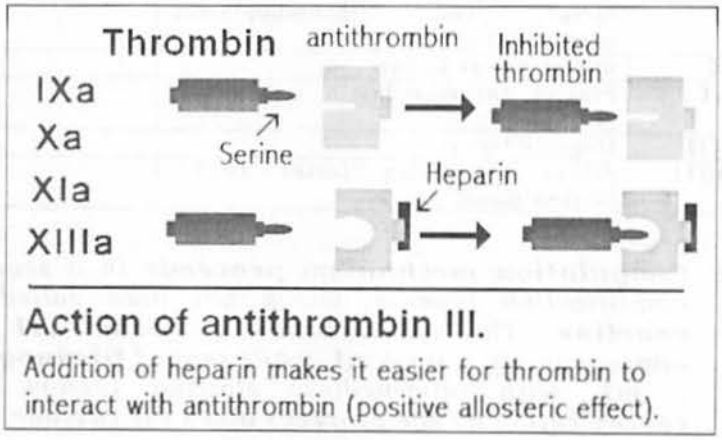
Factor	Common name	Comment
(1) I	Fibrinogen	These factors are usually referred by their common names.
(2) II	Prothrombin	
(3) III	Platelets phospholipids	These are usually not referred to as coagulation factors.
(4) IV	Calcium	
(5) V	Proaccelerin, labial factor, accelerator (AC-) globulin	
(7) VII	Proconvertin, serum prothrombin conversion accelerator (SPCA) cothromboplastin	
(8) VIII	Antihemophilic factor A, antihemophilic globulin (AHG)	
(9) IX	Antihemophilic factor B, Christmas factor, plasma thromboplastin component (PTC)	
(10) X	Stuart-Power factor	
(11) XI	Plasma thromboplastin antecedent (PTA)	
(12) XII	Hegman factor	
(13) XIII	Fibrin stabilizing factor (FSF), fibrinoligase.	

2. ***Coagulation mechanism proceeds*** *in a sequential enzyme amplification process, which has been called the* ***cascade reaction.*** *The concentration of factor* ***XII*** *in plasma is approximately* ***3 ug / ml,*** *while that of* ***fibrinogen*** *is* ***3000 ug / ml,*** *with intermediate clotting factors increasing in concentration as one proceeds down the cascade.*
3. ***Serine protease factors:***
   a) *These are factors II (prothrombin), VII, IX, X, XI, XII and perkallikerein.*
   b) *Serine proteases are those factors (enzymes), which possess serine residues at their active center, and acting by splitting polypeptides.*
4. ***Vitamin K dependent factors:***
   a) *These are factors* ***II (prothrombin) , VII , IX and X .***
   b) *They are synthesized in liver as precursors containing 10-12 glutamic acid residues. These residues are then* ***carboxylated*** *in a reaction requiring vitamin K as a coenzyme to form* ***γ-carboxyglutamate*** *residues which have a high affinity for* ***calcium binding*** *(see vitamin K, part I).*
5. ***Role of thrombin in blood coagulation:*** *It is responsible for activation of the following factors: I (fibrinogen), V, VII, VIII and XIII.*
6. ***Role of ionized calcium (Ca^{2+}) in blood coagulation:*** *It is important for activation of the following factors: II (prothrombin), IX, X and XIII.*
7. ***Role of platelets in blood coagulation:***
   a) *Formation of platelet plug (white thrombus): discussed before.*
   b) *Platelets provide phospholipids on its membrane surface. Platelet phospholipids are also known as platelet factor 3.*
   c) *Platelets have a membrane receptors for factor Va which in turn bind factor Xa.*

E. <u>Inhibitors of coagulation</u>: In normal hemostasis, the concentration of active thrombin must be carefully controlled to prevent spontaneous clots. The natural inhibitors of coagulation

provide mechanism to **limit clotting to the location of tissue injury.** The major inhibitors of coagulation include:

1. **Antithrombin III:** It is the main coagulation inhibitor of plasma:
   a) It inhibits **thrombin, factors IXa, Xa, XIa, and XIIIa.**
   b) Its mechanism of action is enhanced by heparin which binds to specific site on antithrombin III, inducing conformational changes and promoting its binding to thrombin and other factors.



Action of antithrombin III.
Addition of heparin makes it easier for thrombin to interact with antithrombin (positive allosteric effect).

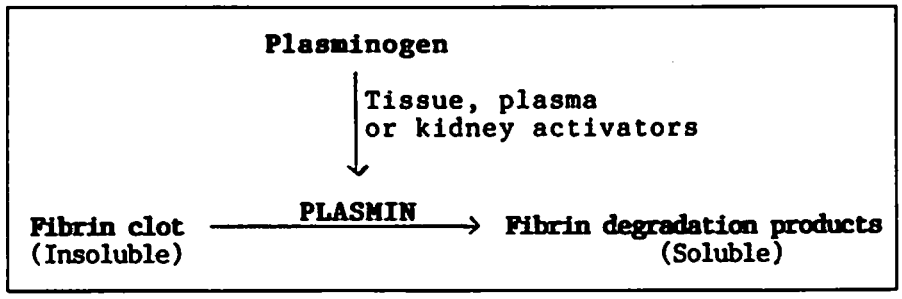
2. **Heparin co-factor II:** Its activity is enhanced by heparin. It **inhibits thrombin.**
3. **α_2-Macroglobulin:** It is one of plasma proteins. It inhibits **thrombin** and **kallikrein.**
4. **Protein C and protein S: protein C** is vitamin K dependent protein, which inhibits factors **Va and VIIIa. Protein S** acts as a cofactor for activation of protein C.

F. Hemophilia:

1. These are a group of inherited diseases in which one of clotting factors is deficient. Patient suffering from hemophilia shows frequent bleeding even from minor traumas. Tests that measure whole clotting time are all prolonged.
2. **Types:**
   a) **Hemophilia A:** is the most common type due to deficiency of factor **VIII.** The disease is an X-chromosome linked disease. It affects only males.
   b) **Hemophilia B** is also present, due to deficiency of **factor IX. Hemophilia C** Von Wilbrand disease.
3. **Treatment of hemophilia** is by repeated blood or plasma transfusion. Factor VIII prepared from pooled donors plasma may also be given. Factor VIII is produced also by **recombinant DNA technology.**

G. Fibrinolysis:

1. This is the **dissolution of clotted blood** after their formation by a blood enzyme called plasmin.
2. Plasmin is present in plasma in an inactive form, which is called plasminogen.
3. Plasminogen is activated by a number of activators, which are derived from tissue, plasma or kidney as urokinase and streptokinase enzymes. These factors are used in treatment of recent blood clots as in myocardial infarction.



H. Anticoagulants:

These are substances that interfere with blood coagulation, either in vivo or in vitro, by removal of any factor of coagulation mechanism. They include:

1. **Citrate** (that binds ionized calcium) and **oxalate** (that precipitates calcium as calcium oxalate).
2. **Defibrination of blood:** Removal of fibrin by stirring by a glass rod.
3. **Heparin:** See the following table.
4. **Dicumarol:** See the following table.
5. **EDTA.**

	Heparin	Dicumarol
Origin	Animal (mast cells).	Plant.
Structure	Proteoglycan.	Similar to vitamin K.
Mode of action	Inhibits thrombin.	Antagonizes vitamin K.
Onset of action	Rapid.	Late.
Duration	Remains for a short time.	Remains for a long time.
Antidote	Protamin sulphate..	Vitamin K.
Site of action	In vivo & vitro .	In vivo only.

Urine

I. Physical properties of urine:

A. Volume:

1. Normally: 800 - 2000 ml/day.
2. **Polyuria** is the urine excretion of more than 2000 ml/day. It is caused by:
   a) **Physiological polyuria** as in high fluid intake (water, tea), and high protein diet (the end product of protein metabolism is urea which causes osmotic diuresis).
   b) **Pathological polyuria** as in diabetes mellitus (glucose causes osmotic diuresis) and in diabetes insipidus due to lack of antidiuretic hormone. (**Note:** diabetes means increased urine volume).
   c) **Oliguria and anuria:** Even under condition of severe water restriction, an individual usually excretes at least 500 ml urine/day. Oliguria is the excretion of less than 500 ml/day. Anuria is the excretion of less than 125 ml / day. They may be caused by:
      1) **Physiological oliguria** as in low fluid intake and at hot weather due to excessive sweating.
      2) **Pathological oliguria or anuria** as in urinary obstruction (by stones or tumor), excessive vomiting and diarrhea or due to shock and hemorrhage.

B. Odor:

1. Fresh urine has an **aromatic** odor.
2. **On standing,** for any length of time, urine gets the **odor of ammonia** due to decomposition of urea by bacteria and release of ammonia.
3. Some diets and medicine may change urine odor.

C. Color:

1. Normally, urine is pale or amber yellow.
2. Dilute urine is pale yellow, while concentrated urine appears almost deep orange.
3. The color of urine is due to 2 pigments; urochrome and urobilin.
4. **Variations of urine color** may result from many metabolic products , drugs and foods.
   a) **Red or red brown** color due to hematuria.
   b) **Yellow-brown or green-brown** due to jaundice.

D. Aspect (appearance):

1. **Normally,** freshly voided urine is **transparent.**
2. **Turbid urine** may be associated with the presence of abnormal constituents e.g. pus (pyuria), red cells (hematuria), chyle (chyluria) and crystals (calcium oxalate, phosphate or urate).

E. Specific gravity (urine relative mass density):

1. Specific gravity (SG), of any liquid **is its density compared with the density of distilled water,** which has a density of 1,000. **(Liquid density / water density).**
2. **Normally,** the specific gravity of urine collected over 24-hours **ranges 1.015-1.025.**
3. The higher the urine specific gravity, is the more the dissolved solids in urine e.g. urea, uric acid, sugar.
4. Urine specific gravity **indicates the concentrating power of the kidney.**
5. **Variations of specific gravity:**
   a) Decreased in cases of dilute urine as in diabetes insipidus.
   b) Increased in cases of concentrated urine as in diabetes mellitus.

F. Urine pH:

1. **Normally, urine pH is acidic (about 6).**
2. Acidity of urine **results from conversion of basic phosphate** (Na_2HPO_3) into **acid phosphate** (NaH_2PO_3) in distal convoluted tubules of the kidney.
3. **After meals,** urine pH becomes less acidic. This is due to the formation of gastric HCl is associated with absorption of more bicarbonate. The latter is then excreted in urine making it alkaline. This process is called **alkaline tide.**
4. **Low urine pH (below 6):** may be associated with:
   a) High protein diet.
   b) Metabolic and respiratory acidosis.
   c) Urinary tract infection by a type of bacteria called: **E.coli.**
5. **High urine pH (above 6):** may be associated with:
   a) High citrus fruits and vegetables.
   b) Administration of some alkalies as sodium bicarbonate.
   c) Potassium depletion, as it leads to alkalosis.

G. Deposits (sediments):

1. Normally, urine contains no visible deposits.
2. Upon centrifugation, one or more of the following deposits may appear by using microscope:

a) **Pus cells** which are dead leucocytes (pyuria): It indicates urinary tract infection.

b) **Red cells** (hematuria).

c) **Epithelial cells:** Squamous epithelium is normally present in female urine. They are derived while urine passing female genital tract. Columnar or transitional epithelium are derived from kidney, ureter or bladder due to a variety of causes e.g. infection.

d) **Parasites and ova:** e.g. bilharzial ova.

e) **Casts:** These are cylindrical structures formed from mucoproteins in the distal convoluted tubules. After formation, they become loose and go down the tubules into the urine. They indicate chronic glomerulonephritis.

f) **Crystals:** as urate, oxalate, and phosphate crystals.

II. *Normal constituents of urine:*

Organic materials	Inorganic materials
Urea	**Sodium (Na^+)**
Ammonia	**Potassium (K^+)**
Creatinine	**Chloride (Cl^-)**
Creatine	**Bicarbonate (HCH_3^-)**
Uric acid	**Phosphate**
Amino acids	**Sulfate**
Proteins	
Sugars	
Others	

A. Nonprotein nitrogenous compounds :

1. **Urea:**
   a) It is **end product of protein metabolism.** It is the main solute in urine.
   b) Normally it ranges **20-40 g/day.**
   c) It is formed in the liver, and excreted by the kidney.
   d) It constitutes about 85% of the total urinary nitrogen.
2. **Ammonia:**
   a) Normally it is about **0.7 g/day.**
   b) Ammonia in urine is derived from deamination of amino acids mainly glutamine:
   c) Formation of ammonia by the kidney is increased in acidosis as in diabetes mellitus. In alkalosis, ammonia is almost absent in urine.
3. **Creatinine and creatine:**
   a) Normally creatinine excretion is about **1.4 g/day,** while creatine excretion ranges **0-0.2 g/day.**
   b) Creatinine (anhydrous creatine) is the end product of creatine metabolism.

c) Creatinine excretion depends on muscle bulk of the individual and not on diet.

4. **Uric acid:**
   a) It is **end product of purine metabolism.**
   b) Normally it is about **0.5 g/day.**
   c) Uric acid is either derived exogenously from diet or endogenously from breakdown of tissue nucleoproteins in liver.
   d) Uric acid is **acidic** because it contains 3 enol groups (C-OH) that can give (H^+) ions.
   e) Uric acid solution is **alkaline in reaction.** This is because uric acid is slightly soluble in water but highly soluble in alkalis. So, the alkaline reaction of uric acid is due to the alkali in which uric acid is dissolved and not uric acid itself.
   f) Uric acid excretion is increased in leukemia, severe liver disease and gout.
   g) **Allanation** is a substance derived from partial oxidation of uric acid in birds. Human urine contains very small amount of allanation.
5. **Amino acids:**
   a) Normally it ranges 150-200 mg/day.
6. **Other nonprotein nitrogenous compounds:** excreted in urine include hippuric acid, indican, purines and coproporphyrins.

B. <u>Proteins:</u> proteins as such are excreted in urine in very small amounts (less than 30 mg/liter).

C. <u>Sugars:</u> 50 % of people excrete 2-3 mg/dl after heavy meal.

D. <u>Other organic constituents</u>: include ascorbic acid, ketone bodies, oxalic acid, phenols as well as some hormones, vitamins and enzymes.

E. <u>Inorganic constituents</u>: as Na^+, K^+, Cl^- HCO^-_3, phosphate and sulfate.

III. *Abnormal constituents of urine:*

A. <u>Sugars:</u>

1. **Glucose:** Normally less than 0.1 gram glucose is excreted per day. Causes of glycosuria (excessive excretion of glucose) are discussed in carbohydrate metabolism, part II.
2. **Fructose:** (fructosuria), **galactose** (galactosuria) and **pentose** (pentosuria): see carbohydrate metabolism, part II.

3. **Lactose (lactosuria):** presence of lactose in urine. It may occur in mothers during pregnancy, lactation and the weaning period.

B. Proteins (proteinuria):

1. Normal urine contains very little amount of proteins **(less than 30 mg/liter). These** are:
   a) **Albumin** and globulins (30%).
   b) **mucoproteins** of renal origin called: **Tamm Horsfall mucoprotein** (70%) .
2. **Microalbuminuria** is the excretion of proteins **(30-200 mg/liter).** It indicates early affection of kidney as in diabetes mellitus. It cannot be detected by ordinary methods and needs special techniques for its detection.
3. **Proteinuria:** It is the presence of protein in urine **(more than 200 mg/liter).** It is characteristic of all acute and chronic kidney diseases:
   a) Most excreted proteins are **albumin (albuminuria)** due to its low molecular weight and higher plasma concentration compared with globulins.
   b) Proteinuria (albuminuria) may be classified into:
      1) **Prerenal proteinuria:** as in heart failure due to renal venous congestion.
      2) **Renal proteinuria:** due to kidney affection (glomerular) as in glomerulonephritis, or tubular as in pyelonephritis).
      3) **Postrenal proteinuria:** due to lower urinary tract affection as inflammation or tumor of urinary bladder.

4. **Other proteins in urine :**
   a) **Bence Jones protein:** It is an abnormal type of globulins (light chains of immunoglobulins), present in urine of patients suffering from multiple myeloma (malignant plasma cells). It is precipitated at 50^0 - 60^0C, dissolved at 100^0C and re-precipitated on cooling.
   b) **Hemoglobin (hemoglobinuria):** It indicates intravascular hemolysis as in malaria and hemolytic anemia.
   c) **Myoglobin (myoglobinuria):** It indicates massive muscle damage as in burns and severe electric shock.

C. Ketone bodies (ketonuria):

1. **Normally**, less than 18 mg of ketone bodies are excreted per day.
2. **Ketonuria** is the presence of ketone bodies in the urine in abnormal concentrations.
3. Ketonuria may occur associated with ketosis in any condition where carbohydrate utilization is impaired e.g. starvation, carbohydrate poor diet and diabetes mellitus.

D. Bilirubin:

1. The presence of bilirubin in the urine occurs in obstructive jaundice and in some stages of toxic jaundice.
2. It gives the urine a dark greenish brown color.

E. Blood (hematuria):

1. It is the presence of blood or intact red cells in urine .
2. It is caused by urinary bilharziasis, glomerulonephritis, and traumatic or malignant diseases.

F. Porphyrins:

1. Normally, trace of coproporphyrins is excreted in urine per day.
2. The presence of excess amount of coproporphyrins or uroporphyrins in urine occurs in patients suffering from **porphyrias.**

IV. *Urinary stones (calculi):*

A. Over 10% of adult males suffer from urinary stones at least once in a life.

B. Urinary stones are almost always composed of substances normally excreted in the urine. These substances for unknown cause are supersaturated, precipitated and form crystals. The crystals are then bound together by a binding substance forming stone.

C. Urinary stones may be classified into :

1. **Simple stone:** consisted only of a single constituent.
2. **Mixed stone:** consisted of two or more constituents.

D. Chemical composition of urinary calculi: The most common substances enter in stone formation are:

1. **Calcium oxalate.**
2. **Calcium phosphate.**
3. **Calcium carbonate.**
4. **Magnesium ammonium phosphate** (triple phosphate).

5. Less commonly stones are formed of:
   a) **Uric acid:** 4-10%.
   b) **Cystine stone:** less than 1%.
   c) **Xanthine stone:** very rare.

E. **Causes of urinary calculi:** There are many factors that may predispose to the formation of calculi:
1. **Change in urine pH:** as in infection of urinary tract, which makes the urine alkaline due to the action of bacteria on urea. Alkalinity causes precipitation of crystals and stone formation.
2. **Disturbance in vitamins:**
   a) **Excess vitamin D** (= hypervitaminosis D): leads to the absorption of excess calcium and causes calcium stone formation.
   b) **Excess vitamin C** (=L-Ascorbic acid): L-ascorbic acid can be converted in human to oxalate, which may lead to the formation of calcium oxalate stones.
   c) **Deficiency of vitamin A**: leads to roughness of the lining epithelium of the urinary tract. This leads to precipitation of crystals and stone formation.
3. **Disturbance in hormones:** as in hyperparathyroidism. It leads to hypercalcuria and formation of calcium stones.
4. **Excess excretion of uric acid:** as in gout. This leads to formation of uric acid stones.
5. **Excess excretion of cystine:** as in cystinuria. This leads to formation of cystine stones.
6. **Excess mucoproteins in urine:** Mucoproteins act as the cement substance that binds the excreted salts to form stone.

Milk

I. *Definition:*

It is the secretion of mammary glands in human and animals. Milk is essential for feeding the infants up to the age of weaning.

II. *Physical properties:*

A. Color:

1. **White due to the presence of fat globules and calcium phosphate.**
2. **Creamy in cow's milk as it contains excess carotene.**

B. pH: 6 - 7.7.

C. Specific gravity:

1. 1032 at 32°C (human milk) and 1028 at 32° C (cow's milk).
2. When milk is skimmed, the specific gravity rises (1033-1037) owing to the removal of the fat (light constituent).

III. *Composition of milk:*

Human milk is the most suitable nutrition for infant feeding. It has the following advantages over the animal milk:

A. Milk proteins: 1.2 g/dl.

1. Milk proteins are less in human than in animal milk.
2. They are casein, albumin, globulin and enzymes.
   a) **Albumin and globulin** (75%) are soluble i.e. easily to digest. They also contain γ -globulins, which give immunity for the baby.
   b) **Casein** (25 %). It is a phosphoprotein of high biological value. It combines with calcium ions to form the insoluble calcium caseinate (milk clot). This prevents the rapid passage of milk from the stomach to intestine and gives the sense of fullness.
   c) **Enzymes:** milk contains many enzymes as proteinase, peroxidase, and catalase.

B. Milk carbohydrate = Lactose 7.0 g/dl.

1. Lactose is more in human than in animal milk.
2. The sweetness of milk is entirely due to lactose, but it is less sweet than ordinary cane sugar; sucrose. This allows the baby to take large amounts of milk without developing nausea.
3. The advantage of lactose over any other sugar is that on hydrolysis it gives glucose and galactose sugars.
   a) Glucose is a good source of energy.
   b) Galactose has a special value for the rapid synthesis of galactolipids.

C. Milk fat: 3.7 g/dl.

1. The same as in animal milk, but human milk contains more unsaturated fatty acids.
2. Milk fats are saturated fatty acids (48%) and unsaturated fatty acids (= essential fatty acids, 52%).
3. Milk contains a little amount of cholesterol and phospholipids.

D. Milk minerals:

Generally, they are less in human than in animal milk.

1. **Iron:** it does not supply the baby needs, but it is more in human than in animal milk, thus anemia in breast feed is less common.
2. **Calcium and phosphorus:** milk is the richest source of calcium and phosphorus. They are present in human milk in optimum ratio for absorption (Ca / P = 2 / 1).
3. **Sodium and potassium:** it is less in human than in animal milk.

E. Milk vitamins: milk contains most of the vitamins.

1. It is very rich in vitamins A and B_2.
2. It is poor in vitamins C, D, and K.

F. Lactoferrin:

It is iron binding protein present in milk, neutrophils and other body fluids. It has **antibacterial action.**

G. Beside the advantage in composition, human milk has also the following additional advantages:

1. Breast milk is supplied at best **suitable temperature.**
2. It is **sterile** and not liable to be contaminated.
3. **Cheaper** than animal milk.
4. **Not liable to adulteration.**
5. **Psychological effect** on both child and mother.

Differences between human milk and cow's milk:

	Human milk	Cow's milk
Proteins:	1.2 g/dl	3.3 g/dl
Casein	0.3 g/dl	2.7 g/dl
Albumin &glob.	0.9 g/dl	0.6 g/dl
Lactose	7.0 g/dl	4.7 g/dl
Lipids	3.7 g/dl	3.7 g/dl
Sat. FA	48%	58%
Unsat. FA	52%	42%
Minerals	Less	More
Vitamins	More	Less

IV. *Humanization of cow's milk:*

A. Definition: This is a process by which cow's milk is made to be as near as human milk, to suit the infant's needs.

B. Aim: The aim of humanization is to decrease the concentration of casein which if present in high concentrations, forms a dense clot in the infant's stomach and leads to vomiting.

C. Steps:

1. **Pasteurization of milk:** It is sterilization of milk by heating it to 60°C for 30 minutes (or 70°C for 15 minutes) followed by rapid cooling. Milk is then left in cool place for sometimes to allow the fat to concentrate at the surface.
2. **Dilution:** the milk is halved diluted with boiled water to decrease its contents of protein and minerals.
3. **Addition of lactose:** Lactose is added to raise the carbohydrate content.
4. Iron, vitamin C and vitamin D may be added.

V. *Milk at different times of lactation:*

A. *Colostrum:*

1. **Definition:**
   It is the yellowish fluid secreted by the mammary gland during the ***first week of lactation.***
2. **It differs from mature milk in:**
   a) It is yellow in color due to the presence of excess carotenes.
   b) It contains more protein and **is especially rich in gamma globulins**, which supply infant with **antibodies** (give immunity against diseases).
   c) It contains more minerals and vitamins than mature milk.
   d) It contains less fat and less carbohydrate.
3. **Functions of Colostrum:**
   a) **It contains antibodies, which give immunity for the baby.**
   b) **It stimulates intestinal movement, so has laxative action.**

B. Intermediate milk:

It is the milk secreted during the ***first month*** of lactation except the first week.

C. Mature milk:

It is the milk secreted during the ***first year*** of lactation except the first month.

D. Late milk:

1. It is the milk secreted ***after the first year*** of lactation.
2. It contains less protein, lipids, vitamins and more minerals than mature milk; therefore it is less sweaty and helps weaning.

E. Witch's milk:

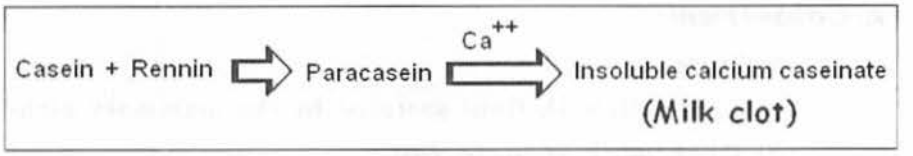
1. It is the fluid secreted by the mammary gland of the infant during the first days of life.
2. It is due to the effect pf placental hormones on the mammary gland of the infant.

	Colostrum	Mature milk
Color	Yellowish as it contains excess carotene	White
Consistency	Thick	Thin
Reaction	Alkaline	Neutral
Proteins	1.5 g/dl and contains excess gamma globulins	1.2 g/dl
Carbohydrates	Less	7 g/dl
Lipids	Less	3.7 g/dl
Minerals	More	Less
Trypsin inhibitor	Present	Absent

VI. *Milk Processing:*

A. Milk clotting (cheese formation):

1. This is the enzymatic precipitation of milk casein by rennin enzyme.



2. Calcium paracaseinate is insoluble and precipitated with fat and fat soluble vitamins forming the milk clot or cheese.
3. Rennin enzyme is usually obtained from the fourth stomach of young calves.
4. **Milk whey:** it is the yellowish green fluid left after milk clotting. It contains lactalbumin, lactglobulins and all water soluble vitamins.

B. Milk curdling or souring (yogurts formation):

1. It is the precipitation of casein by lowering milk pH to 4.6 (isoelectric point of casein).
2. **Milk curdling can be produced by:**
   a) Addition of **acids** e.g. acetic acid.
   b) Action of certain bacteria called **lactobacillus acidophilus**. Such bacteria ferment lactose to lactic acid.

C. Skimmed milk:

It is milk almost contains no fat (only 1% fat).

D. Cottage cheese:

It is the cheese formed from skimmed milk.

E. Sterilization of milk:

1. It is prepared by heating milk at 120°C for 20 minutes to kill all bacteria.
2. Its taste is altered and vitamins C and B1 are destroyed.

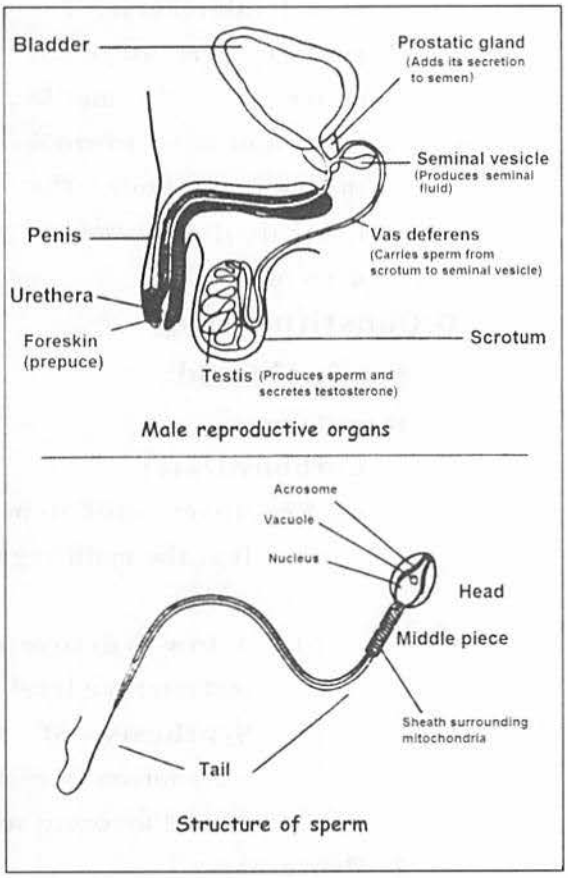
F. Dried milk:

1. This can be done by lyophilisation i.e. centrifugation of milk at high speeds at very low temperature. This removes all water

leaving milk as a powder. This method preserves the biological properties of milk components.

Semen, CSF, and other body fluids

I. *Semen (seminal fluid):*



A. <u>Seminal fluid</u>: is a milky mixture of spermatozoa and secretions of epididymis, seminal vesicles and prostate.

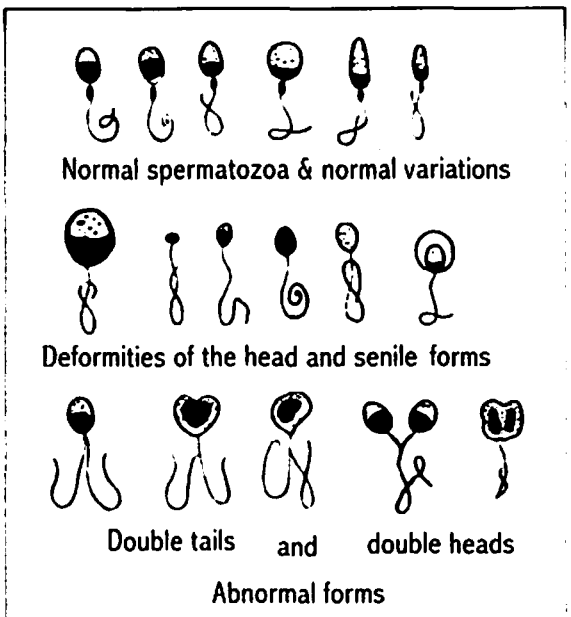
B. <u>Spermatozoa</u>: are produced in the testes: **(spermatogenesis).**

1. It requires **FSH** for its initiation, and high local **testosterone** for its maintenance.
2. Spermatozoa are consisted largely of nucleoprotein. Each spermatozoon is consisted of head, middle piece (neck) and tail.
   a) **Head:** contains the **nucleus** (which contains a haploid number of chromosomes) and **acrosome** (which contains peptidase and hyaluronidase enzymes, which are very important for penetration and fertilization of ovum.
   b) **Middle piece (neck):** contains mitochondria.
   c) **Tail:** which consists of a protein similar to myosin fibrils. Tail is responsible for sperm motility.

C. <u>Characters of seminal fluid:</u>

1. **Volume: 3-5 ml** (60% from seminal vesicle and 20% from prostate).
2. **Number (sperm count):** The average number is **60-120 millions/ml.** The lower normal limit of number is **10 millions/ml.**
   a) Sperm count below 10 millions is called: **oligospermia.**
   b) Absence of sperms is called: **azospermia.**
3. **Shape:** Normally 80% of sperms are normal. About 20 % show abnormal shapers (see figure).

4. **Motility:** The speed of sperm movement is **3 mm/minute.** It can reach the oviduct within **30-60 minute,** after sexual intercourse. The average percentage of active motile spermatozoa is **70-90%** in the first hour. The lower limit of normal is **40%** motile.



Normal and Abnormal Forms of Sperms. Oraby

D. Constituents of seminal fluid:

It contains:

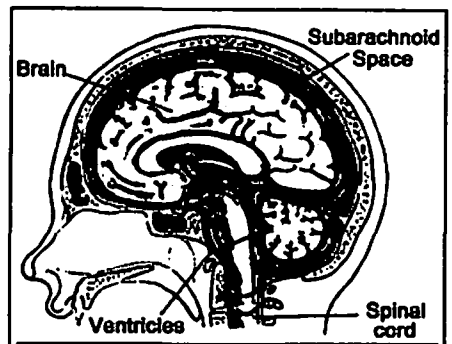
1. **Carbohydrate:**
   - *a)* **Fructose:** 200-600 mg/dl.
     1) It is the main sugar in semen. It is used as a nutrient for sperm.
     2) **A low fructose concentration** is the result of a low testosterone level or seminal vesicle insufficiency.
     3) **Synthesis of fructose in testis:** see fructose metabolism in carbohydrate metabolism, part II.
   - *b)* Citrate and ascorbic acid (derived carbohydrate).
2. **Proteins:**
   - *a)* **Fibrinogen:** This causes rapid clotting of seminal fluid after ejaculation.
   - *b)* **Fibrinolysin:** This causes liquefaction of clotted fluid within 20 minutes after ejaculation.
   - *c)* **Spermine and spermidine:** These are polyamines secreted by the prostate (see protein metabolism). They give semen its characteristic odor.
   - *d)* **Enzymes:** as acid phosphatase, ATPase, hyaluronidase.
3. **Lipids:**
   - *a)* **Prostaglandins**: produced by prostate and cause uterine contractions to help the uptake of semen by the uterus.
   - *b)* **Phospholipids and cholesterol.**
4. **Minerals :**
   - *a)* zinc is very important mineral in semen. Zinc deficiency is associated with hypogonadism and sterility.

b) Seminal fluid is also rich in potassium, calcium and magnesium.

II. *Cerebrospinal fluid (CSF):*

A. Formation:

1. **CSF is produced** partly by ultrafilteration of plasma and partly by active secretion.
2. The choroid plexuses of brain ventricles secrete CSF.
3. It fills the **ventricular system** of the brain and the **subarachnoid space** surrounding the brain and the spinal cord.
4. CSF volume is **60-160 ml.** CSF is constantly produced **(about 250-750 ml/day).** This means that CSF is also continuously reabsorbed from subarachnoid space into the blood. If absorption is impaired (e.g. after bacterial meningitis, or subarachnoid hemorrhage) CSF volume is increased and CNS pressure rises.



Relationships of ventricles and subarachnoid space with rest of brain.

B. Functions of CSF:

1. To provide a fluid cushion to **protect the brain and spinal cord** from mechanical injury caused by any sudden movement of the body.
2. **To carry nutrients** to the brain and spinal cord and remove waste substances.
3. **To maintain a constant pressure** inside the head and around the spinal cord.

C. Characters and composition of CSF:

1. CSF is obtained by **lumber puncture.**
2. Characters: **volume: 60-160 ml.** Clear, colorless fluid with **pH: 7.4.**
3. It is similar in composition to plasma, but differs in concentrations. It contains:
   a) **Glucose:** 45-70 mg/dl.
   b) **Protein:** 15-40 mg/dl (80% albumin 20% globulin).
   c) **Lipids:** Cholesterol is very low.
   d) **Minerals:** It contains chloride, magnesium, potassium, sodium, calcium and phosphorus. Chloride concentration is higher than that of plasma.
   e) **Non protein nitrogenous compounds:** as urea.

f) **Others:** as hormones, enzymes and vitamins.

g) **White blood cells:** Normally CSF contains up to 5 lymphocytes / mm^3.

4. **Alterations in CSF characters** and / or composition may occur in certain diseases e.g. in bacterial meningitis, the proteins are markedly increased, glucose and chloride are decreased, and the volume is markedly increased.

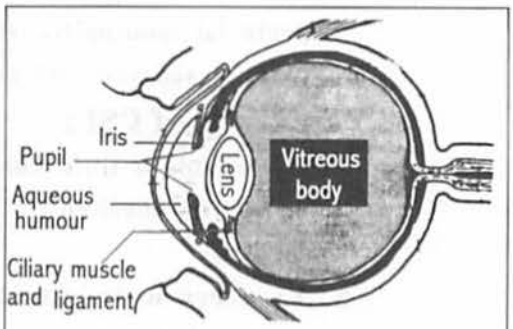
III. *Aqueous humor and vitreous humor:*

A. Aqueous humor:

1. It is the watery fluid that fills the anterior and posterior chamber of the eye.
2. It is formed by **the ciliary processes**, passes through the posterior chamber and the pupil into the anterior chamber where it is reabsorbed into the venous system.
3. It is very **similar in composition to CSF.**

B. Vitreous humor (vitreous body):

1. It is present in the posterior part of the eye ball (behind the lens).
2. It is gel-like substance.
3. It is rich in mucoproteins.
4. It supports eye shape.



Cross-section of the eye as viewed from the side.

IV. *Sweat and tears:*

A. These are hypotonic solutions containing mainly Na^+ and Cl^- .Sweat also contains traces of urea and K^+.

B. Lysozyme, an enzyme that has an antibacterial action, is present both in sweat and tears.

V. *Lymph:*

A. Lymph is an interstitial fluid, which has passed into the lymph vessels. It is formed by filtration of plasma through the capillary wall.

B. **Composition:** It is similar to plasma, but proteins in lymph are lower than plasma. Lymphocytes are added to lymph while passing through the lymph nodes.

C. The lymph of intestine is called: **chyle.**

VI. *Amniotic fluid:*

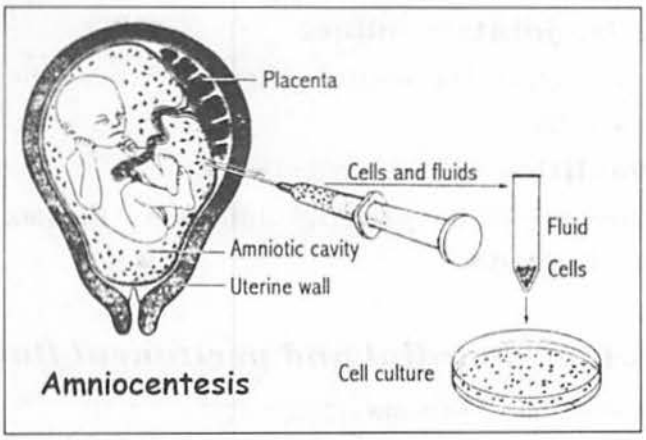
A. It is the fluid contained inside the **amniotic sac** in which the embryo is free to move and protected against mechanical injury.

B. Amniotic fluid is similar in composition to the extracellular fluid and contains:

1. Undissolved material from the fetal urine and respiratory secretions.
2. Secretions from the placental membrane .

C. **Volume** of amniotic fluid ranges **450 - 1500 ml.**

D. **Amniocentesis** is the Trans abdominal aspiration of fluid from the amniotic sac for biochemical and cytological analysis.



E. **Analysis of the amniotic fluid** and cells helps in diagnosis of many intrauterine fetal diseases and abnormalities:

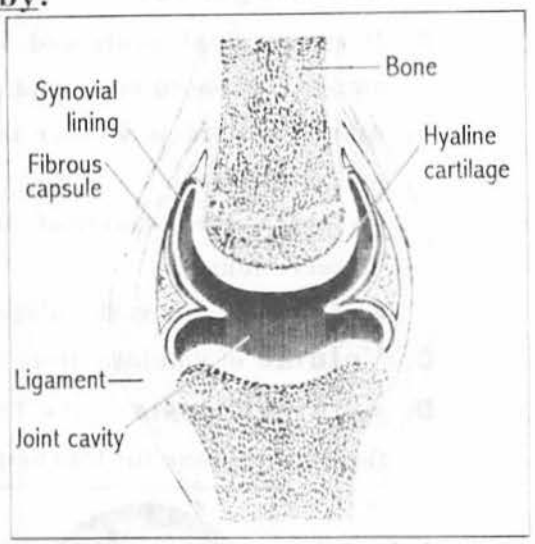
1. **Measurement of the lecithin/sphingomyelin (L/S) ratio** indicates maturation of fetal lungs. Ratio 4/1 indicates mature lungs and ratio less than 4/1 indicates immature lungs.
2. **Measurement of bilirubin** in amniotic fluid indicates the degree of fetal red blood cell destruction and the severity of anemia as in Rh incompatibility.
3. **Measurement of alpha fetoprotein** (a globulin produced by the fetal liver between the 6^{th} and 32^{nd} weeks of pregnancy) indicates some fetal abnormalities as neural tube defects.

VII. *Synovial fluid:*

A. Synovial fluid is present in joint cavities and tendon spaces. It contains high percent of hyaluronic acid.

B. Synovial fluid is produced by:

1. Ultrafiltration of plasma.
2. β-Synovial cells which produce hyaluronic acid.



C. The function of synovial fluid are:

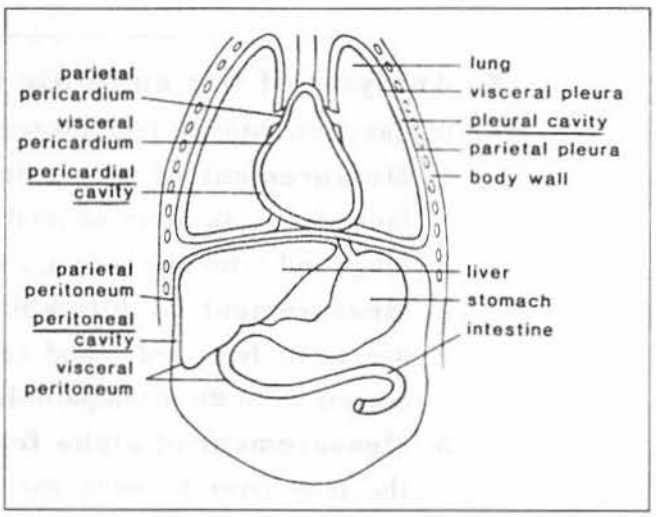
1. It minimizes the friction between bones during movement or weight bearing.
2. It provides nutrition for cartilage.

D. Normally, joints produce 0.15 - 3.5 ml clear, transparent, slightly viscous synovial fluid that can produce clot.

E. Abnormalities in volume, transparency, or viscosity occur in joint diseases as in bacterial infection, rheumatic fever, or rheumatoid arthritis.

VIII. *Pleural, pericardial and peritoneal fluids:*

A. These are a **serous effusion** produced by plasma ultrafiltration within the pleural, pericardial, and peritoneal cavities. The word serous is derived from serum.



B. Normally, there is less than 20 ml in pleural cavity, from 20 to 50 ml in pericardial sac and less than 100 ml in peritoneal cavity.

C. The function of these fluids is to lubricate the parietal and visceral tissues during organ movement.

D. Abnormal accumulation of fluids in such cavities occurs in many primary and secondary diseases affecting corresponding organs. Accumulation of fluid in peritoneal cavity is called: **ascites.**

Chapter 4 *Immunochemistry*

I. *Introduction:*

The body defends itself from infections and other foreign substances in a number of ways:

A. <u>Mechanical barriers</u>: which protects against external organisms:

1. **These are skin, hair, tears and mucous secretions.**
2. **They contain enzymes that kill bacteria.**

B. <u>Leucocytes</u>:

They are mobilized very fast but not adequate in all cases.

C. <u>Immune system</u>: It takes time to develop effectively, especially when the body sees the organism for the first time.

1. The immune system is comprised of 2 components, which are **cellular immunity** and **humoral immunity.**
2. This chapter will discuss humoral immunity where immunological reactions can occur between two types of substances, immunoglobulins and antigens.

II. *Immunoglobulins (Antibodies)*

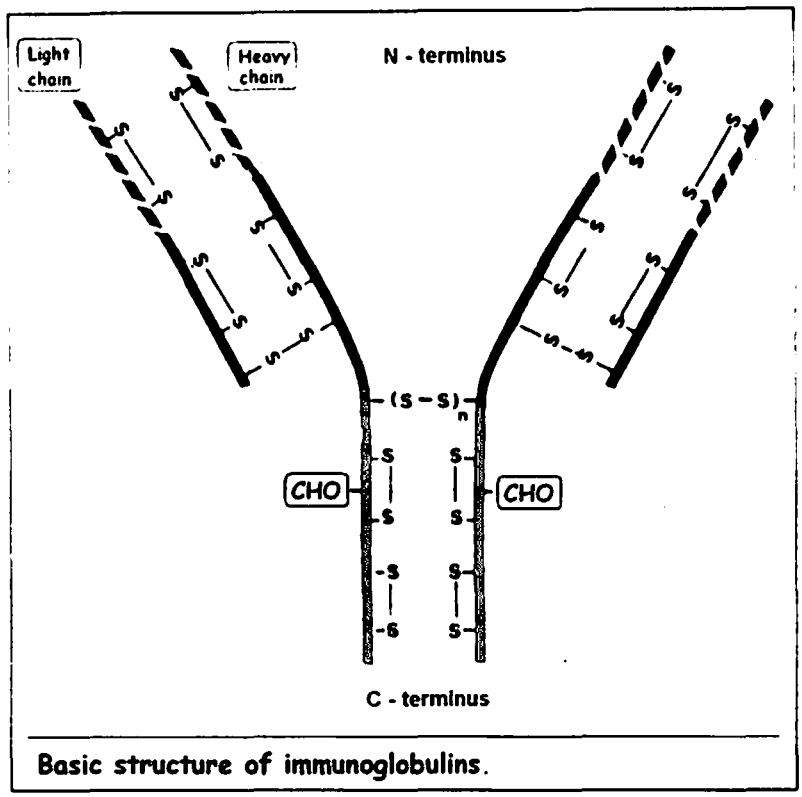
A. <u>Definition:</u>

1. **Immunoglobulins (Ig), (= antibodies):**
   - *a)* These are a group of proteins (gamma globulins) produced by the body (from B lymphocytes and plasma cells) in response to the presence of foreign substances.
   - *b)* They are 5 basic types: **M, A, G, E** and **D.**
   - *c)* The human body can produce antibody molecules, directed to about 1 million different kinds of antigens.
   - *d)* All immunoglobulins have a similar basic structure.

B. <u>Basic structure of immunoglobulins</u>:

1. The basic unit of all immunoglobulin molecules consists of 4 polypeptide chains linked by disulphide bonds:
   - *a)* 2 polypeptide chains are light (L) chains, of low molecular weight.
   - *b)* 2 polypeptide chains are heavy (H) chains, of high molecular weight.

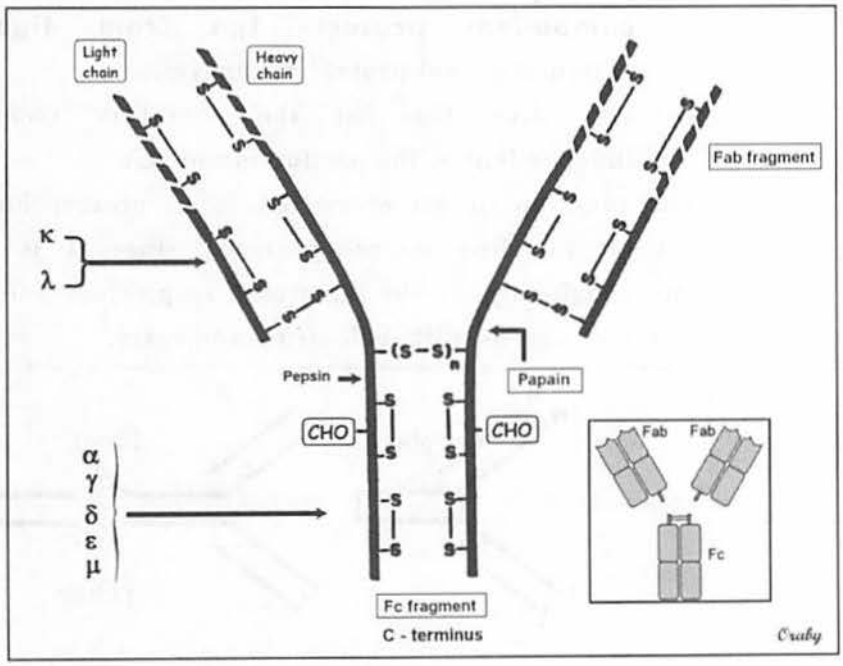
2. Both heavy and light chains have 2 regions:
   a) **C-terminal constant regions**: having a constant amino acid sequences within a class or type.
   b) **N-terminal variable (v) regions**: with considerable variation in amino acid sequence from molecule to another.



Basic structure of immunoglobulins.

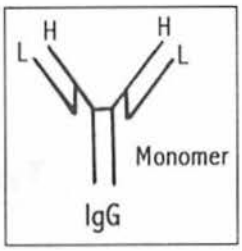
3. Immunoglobulin G (IgG) is composed of one basic unit and it is called: monomer, IgA is composed of 2 units (dimer) and IgM is composed of 5 units (pentamer).
4. **Antigen-binding sites on immunoglobulins:** This is the part of antibody molecule, which combines with antigens. It is formed by a few amino acids in the variable (v) region of H and L chains (dotted lines), at N-terminal ends. Thus there are 2 binding sites per immunoglobulin molecule, a property known as divalency.
5. **Classes of L and H chains:** Several classes of L and H chains have been described:
   a) There are only 2 major types of L chains in man, **the kappa (κ) and lambda (λ) chains.** Either type of light chain can be associated with each of the heavy chain classes. Approximately 70% of the human immunoglobulin molecules carry κ light chains and 30% carry λ light chains.
   b) The H chain is unique to the class. It is termed gamma (γ) chain In IgG, alpha (α) chain in IgA, mu (μ) chain in IgM, delta (δ) chain in IgD and epsilon (ε) chain in IgE.

6. Immunoglobulins contain carbohydrate (CHO) residues (2% to 12 %). These are mannose, galactose, fructose, N-acetyl neuraminic acid or glucosamine. They are attached to polypeptide chains.
7. **Papain enzyme** cleaves immunoglobulins into 2 antibody fragments [FAB] fragments (which contain variable fragments) and one constant fragment [Fc] (which contains constant fragment)



C. Types of immunoglobulins:

1. **Immunoglobulin G (IgG):**



   a) It forms 80% of the serum immunoglobulins.
   b) It is the **only** immunoglobulin, which has the ability to cross the placenta.
   c) It is the major immunoglobulin during the **secondary immune response** with long half-life if compared with other immunoglobulins.
   d) It is of low molecular weight formed of **one unit only**, and contains 2-4 % carbohydrate.
2. **Immunoglobulin A (IgA):**
   a) It is present in the **serum as monomer**, but it is present in high concentrations in **body secretions as dimer.**
   b) In dimeric IgA, a small polypeptide chain called a j chain linked to Fc regions joins the molecules.

c) IgA cannot cross the placenta.

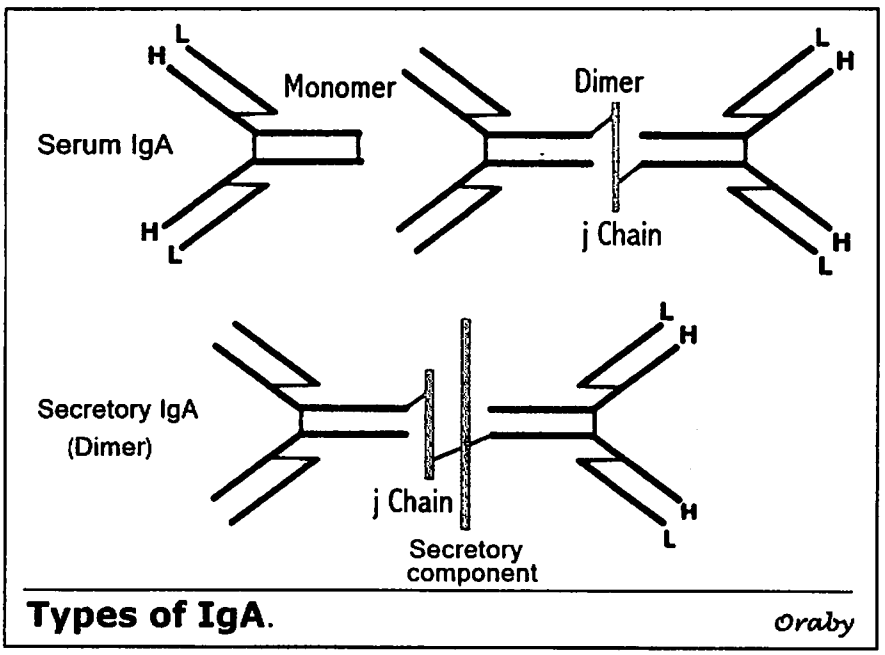
d) IgA contains **5-10 %** carbohydrate.

e) The IgA produced in the intestinal wall may either: diffuse into the blood stream or binds to a glycoprotein called **a secretory component** (or secretory piece) to form a complex molecule called **secretory IgA,** which pass directly into intestinal lumen.

1) Secretory IgA is the predominant immunoglobulin in the intestinal and other secretions. Its **secretory component protects IgA from digestion** by gastrointestinal proteolytic enzymes.

2) The production of the secretory component is independent of the production of IgA.

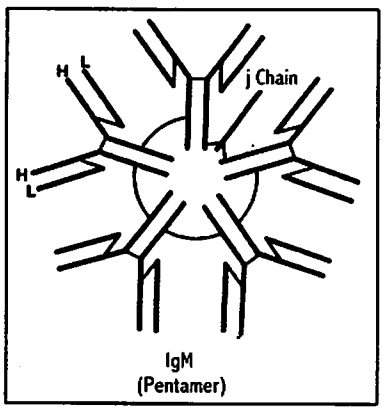
f) The function of secretory IgA is to protect body surface against invading microorganisms, since it is the major immunoglobulin in the intestinal, respiratory and urogenital tracts as well as milk, colostrum and tears.



Types of IgA. Oraby

3. **Immunoglobulin M (IgM):**

a) IgM is present in serum and has the highest molecular weight amongst all immunoglobulins as it is formed of 5 basic subunits (pentamer).



b) IgM subunits are joined together by disulfide bonds in a circular fashion to form star.

- A small cysteine rich polypeptide chain called j chain binds two of the subunits to complete the circle.

c) IgM is the major immunoglobulin during the **primary immune response** i.e. it is the first of the antibodies which act on introduction of a foreign antigen into the plasma. Its presence indicates **recent infection**.

d) IgM cannot cross the placenta.

e) IgM contains 10% carbohydrate.

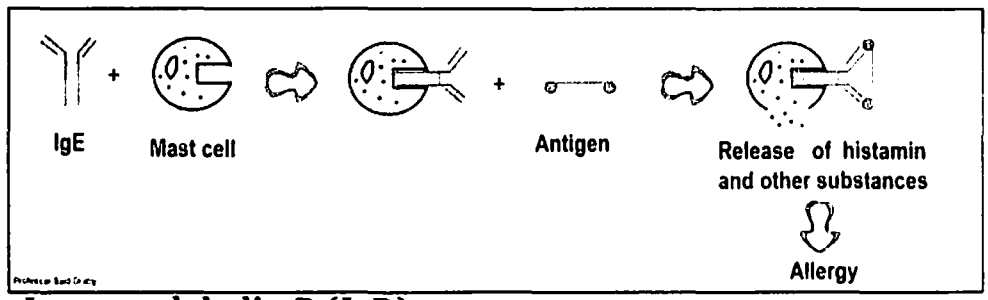
f) IgM has a relative short half-life.

4. **Immunoglobulin E (IgE):**

a) It is present in very low concentration in serum and has the shortest half life (2 – 3 days).

b) It is largely responsible for immunity against parasites.

c) IgE (through its Fc regions), in the presence of antigen, binds to mast cells and basophils, causing release of histamine and other substances from mast cell. These substances result in allergic manifestations.



5. **Immunoglobulin D (IgD):**

a) It is present in a very low concentration in serum.

b) It cannot cross the placenta.

c) IgD is a cell membrane immunoglobulin found on the surface of B-lymphocytes. It serves in this location as a specific antigen receptor.

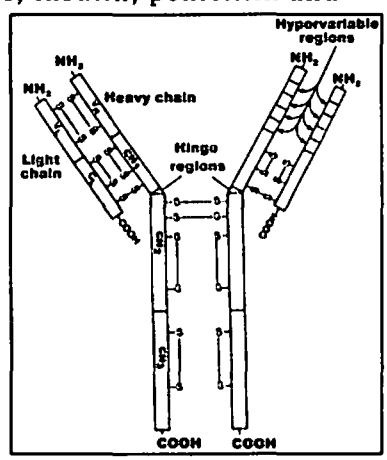
d) IgD has activity against thyroid tissue, insulin, penicillin and diphtheria toxoid.

D. Genes of light and heavy chain:

Both light and heavy chains are products of multiple genes:

1. **Each immunoglobulin light chain is the product of at least 3 separate structural genes, these are:**

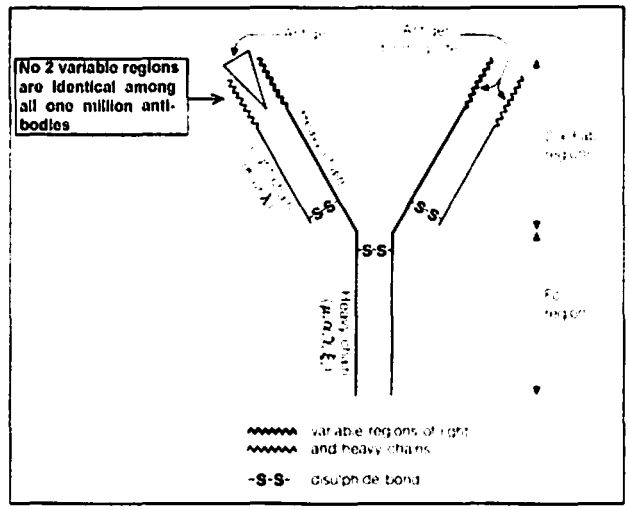
a) A variable region (V_L) gene.



b) A joining (J) region gene (which has no relationship to the (J) chain of IgA or IgM).

c) A constant region (C_L) gene.

2. **Each immunoglobulin heavy chain is the product of at least 4 different genes:**
   a) A variable region (V_H) gene.
   b) A diversity region (D) gene.
   c) A joining (J) region gene.
   d) A constant (C_H) gene.



3. **Antibodies diversity:** There are more than 1 million antibodies. These are derived from only 5 basic types (IgG, IgM, IgA, IgE and IgD). This can be explained by the fact that all are sharing the same **constant regions** (within a class or type) but differ in **variable regions** of both L & H chains i.e. no 2 variable regions are identical.
4. The same variable light and heavy chains may combine with the constant chains of immunoglobulin G, A and M. This means that the 3 classes can be directed and combined to the same antigen.

E. Over and underproduction of immunoglobulins:

1. **Overproduction (hypergammaglobulinemia):**
   a) It is due to increased synthesis and amounts of immunoglobulins.
   b) **It is of two types:**
      1) **Diffuse hypergammaglobulinemia: where** all immunoglobulin classes are increased.
      2) **Discrete hypergammaglobulinemia (paraproteinemia):** where a single immunoglobulin or immunoglobulin fraction (e.g. light chain) is increased. Two categories are present:
         i- **Malignant paraproteinemia:** e.g. multiple myeloma.
         ii- **Benign paraproteinemia.**

2. **Underproduction (hypogammaglobulinemia):**
   It may be congenital or acquired leading to immunodeficiency and recurrent infections.

III. *Antigens (= immunogens)*

A. Definitions:

1. **Antigens** are substances when introduced into the body will stimulate an immune response i.e. antibodies production.
2. **Haptens** are small molecules that cannot by themselves induce antibody formation but can do so when covalently linked to larger molecules.

Hapten + Lymphocytes → No Antibodies
Hapten + Protein + Lymphocytes → Antibodies against this hapten

B. Properties of antigens :

1. **High molecular weight:**
   Proteins with molecular weights greater than 100,000 are the most potent antigens.
2. **Foreign to the body:**
   a) Under normal conditions, tissues or fluids of the body can be recognized by immune system as self (i.e. own tissues), and do not stimulate immune response.
   b) If foreign substances are introduced in the body, immune system recognizes them, as non-self and immune response will occur.
3. **Structural complexity:**
   a) **A molecule must possess a certain degree of complexity to be antigenic.**
   b) **Immunogenicity increases with structural complexity.**

C. Chemical nature of antigens:

1. Proteins, polysaccharides and other synthetic polymers are good antigens.
2. Lipids are not antigenic unless they are combined with proteins or polysaccharides.

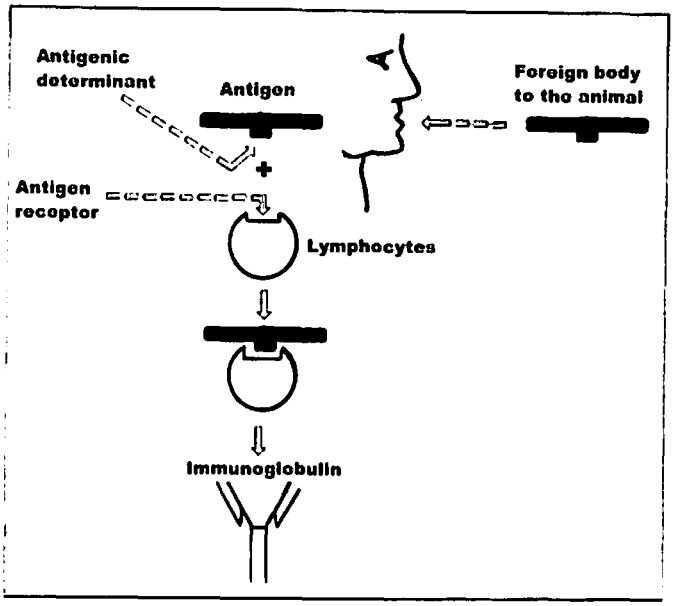
D. Genetic constitution of the animal:

1. Antigen which can induce an immune response in a particular animal have no effect on another animal. This depends on the genetic constitution of the animal.

2. **Initiation of immunoglobulins production** requires binding of the antigen to the lymphocyte surface.

a) **Antigen receptors:**
These are the sites on the surface of lymphocytes that bind the antigen.

b) **Antigenic determinant:**
This is the portion of antigen that binds with the antigen receptors.



3. **Immunopotency:**
It Is the capacity of the region of the antigenic determinant to induce the formation of specific antibodies.

Comparison between different types of immunoglobulins (Ig):

	IgG	IgA	IgM	IgE	IgD
% Of serum content	**80%**	**20%**			
Crossing placenta	**The only Ig that can cross it**	-	–	–	–
Number of basic structural units	Monomer (1)	Monomer (1) or Dimer (2)	**Pentamer (5)**	Monomer (1)	Monomer (1)
Type of heavy chain	Gamma (γ)	Alpha (α)	Mu (μ)	Epsilon (ε)	Delta (δ)
Type of light chain	Kappa (κ) or Lambda (λ)	Kappa (κ) or Lambda (λ)	Kappa (κ) or Lambda (λ)	Kappa (κ) or Lambda (λ)	Kappa (κ) or Lambda (λ)
Carbohydrate content	2-4%	5-10%	10%	10-12%	2%
J-chain	-	+	+	-	-
Secretory component	-	+	-	-	-
Function	The major immunoglobulins **during the secondary immune response.** Their presence indicates old infection or immunization	The major immunoglobulins In the intestinal, respiratory and urogental tract. They protect body surfaces against invading microorganisms.	The major immunoglobulins **during the primary immune response.** Their presence indicates recent infection	Mainly responsible for immunity against parasites.	?
Half life	Long	Short	Short	Shortest	Short

Chapter 5 *Minerals and water metabolism*

I. *Classification:*

According to the body needs, minerals may be divided into 2 groups:

A. Macrominerals:

1. They are required in amounts greater than 100 mg/day.
2. They include 6 elements: calcium, phosphorus, magnesium, sodium, potassium and chloride.

B. Microminerals (trace elements):

1. **They are required in amounts less than 100 mg/day.**
2. **They include 10 elements: chromium, cobalt, copper, fluoride, iodine, iron, manganese, molybdenum, selenium, zinc and silicon.**

Macrominerals

I. *Calcium*

A. Sources:

1. Milk and milk products (the richest sources).
2. Beans, leafy vegetables and egg yolk.

B. Absorption:

1. Site: upper small intestine.
2. Absorption of calcium is active process and requires calcium-binding protein present in the intestinal mucosal cells.
3. **Absorption is regulated by:**
   a) **Vitamin D:** [1,25 dihydroxycholecalciferol (=calcitriol)]: through the formation of calcium binding protein **(calbindin).**
   b) **Parathyroid hormone: Increase calcium absorption** through the conversion of vitamin D to 1,25 dihydroxy-cholecalciferol in the kidney. (See vitamin D, part I).
4. **Factors affecting calcium absorption:**
   a) **Factors promoting calcium absorption:**
      1) **High protein diet:** Amino acids form with calcium a soluble calcium salts which are easily absorbed.

2) **pH:** an acidic pH in the upper small intestine is essential for calcium absorption.
3) **High dietary lactate or citrate:** that form soluble salts with calcium.

b) **Factors inhibiting calcium absorption:**

1) **High dietary phosphate, oxalate and phytate:** which form insoluble salts with calcium.
2) **Alkalinity:** excessive alkali intake (as during treatment of peptic ulcer) decreases calcium absorption.
3) **Impaired fat absorption:** fatty acids form insoluble calcium soaps with calcium.

C. Body calcium: *Calcium is the most abundant mineral in the body (about 1200 grams).*

Body calcium:
* **99%: Bones.**
* **1% : Plasma and other tissues**

1. **99% present in bones and teeth:** *in the form of hydroxyapatite:* $3\ Ca_3(PO_4)_2.Ca(OH)_2$ *i.e. 3 molecules of calcium phosphate and one molecule of calcium hydroxide.*
   a) Calcium salts in bones are not inert. They are in a constant state of turnover in skeleton being deposited in sites of bone formation and released at sites of bone resorption. In adult male about ***700 mg*** *calcium enter and leave bones each day.*
   b) Calcium in bones acts as a reservoir, which helps to stabilize calcium ions in plasma and extracellular fluid.
   c) Parathyroid hormone and active vitamin D stimulate osteoblasts while estrogen hormone inhibits osteoclasts. Thus after menopause ➔ ↓ estrogens ➔ ↓ bone mass (osteoporosis).
2. **1 %:** *of calcium is present in body fluids and other tissues.*

D. Plasma calcium:

1. **Level:** Plasma calcium level ranges from: **8.5 - 10.5 mg / dl.**
2. Blood calcium lies entirely in the plasma (No calcium in RBCs).
3. **Forms:** Plasma calcium is present in 2 forms; ionized and nonionized.
   a) **Ionized (diffusible): (50 %)**
   - It is the active fraction. Its deficiency causes tetany.

b) **Non ionized: (50 %)**

- **(Diffusible)** Complexes with organic ions e.g. citrate: **(5 %).**
- **(non diffusible)** It is bound to protein mainly albumin **(45%).** Its deficiency occurs with conditions of hypoproteinemia and causes no tetany.

4. **Factors affecting plasma calcium** (calcium homeostasis):

a) **Hormonal regulation:** Three hormones are concerned with regulation of blood calcium. These are parathyroid hormone, active vitamin D (calcitriol) and calcitonin.

1) **Parathyroid hormone (PTH):** It increases plasma calcium level through:
   - i- Mobilization of calcium from bones (bone resorption).
   - ii- **Absorption** of calcium from intestine (through conversion of vitamin D into calcitriol "active vitamin D" in the kidney).
   - iii- **Reabsorption** of calcium by renal tubules i.e. ↓ Ca^{2+} excretion in urine and ↑ phosphate excretion.

2) **Calcitriol: 1,25 dihydroxycholecalciferol:** It increases blood calcium level through:
   - i- Absorption of calcium from the intestine.
   - ii- Reabsorption of calcium by renal tubules.
   - iii- Mobilization of calcium from bones.

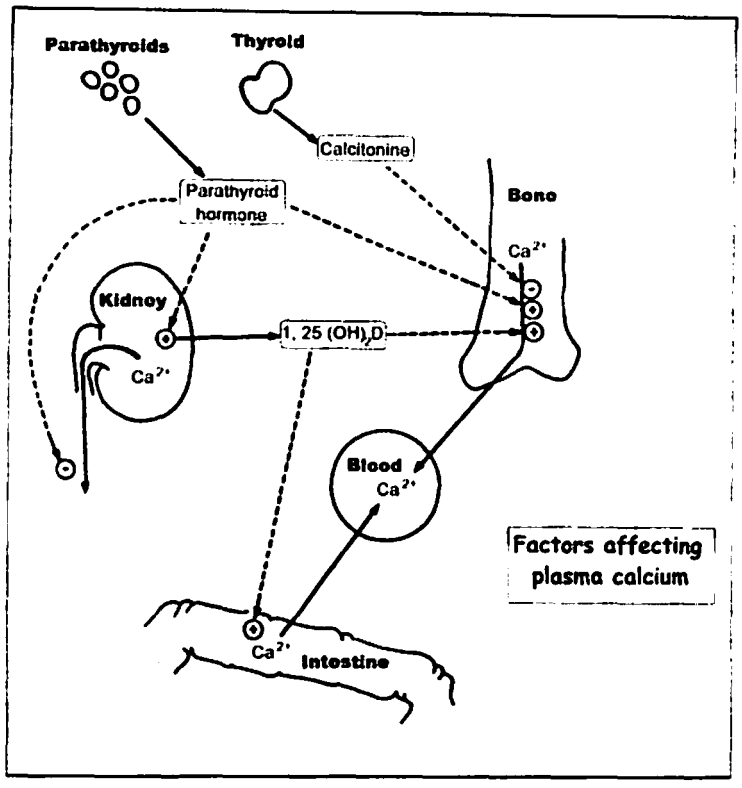
3) **Calcitonin:**
   - i- It is a hormone that is secreted by the parafollicular or "C" cells of the thyroid gland.
   - ii- It is released in response to hypercalcaemia and causes decrease of blood calcium level through inhibition of calcium mobilization from bones, or increasing calcium deposition in bones.

b) **Other factors:**

1) **Solubility product:** Normally Ca/P ratio must be constant. Ca x P in children is 50 and in adults are 40. If plasma phosphate increases (as in renal failure) the plasma, calcium decreases to keep the ratio constant.

2) **Blood pH:** Ionization of calcium occurs at normal blood pH, 7.4. The more the acidosis of the blood pH, the more formation of ionized calcium.

3) **Plasma proteins:** In cases of hypoproteinemia, the non-diffusible calcium decreases.



E. Functions of calcium:

1. Calcification of bones and teeth.
2. Regulation of transmission of nerve impulses.
3. Regulation of contraction of muscles.
4. Decrease of neuromuscular excitability. Deficiency of ionized calcium leads to tetany.
5. Cardiac conduction.
6. Calcium acts as a second messenger for hormonal action by acting together with calmodulin and cAMP.
7. Blood and milk clotting.
8. Maintenance of cell membrane permeability.
9. Activation of certain enzymes e.g. pyruvate kinase.

F. Excretion:

1. Most of calcium excretion is eliminated with feces.
2. Small amount of calcium is excreted in urine (about 200 mg/day).

G. Requirements:

1. Adult men and women: 800 mg/day.
2. Children, pregnant and lactating women: 800-1200 mg/day.

H. Alterations of plasma calcium:

1. **Hypercalcemia:**
   - *a)* **It is caused by:**
     1) **Primary hyperparathyroidism:** usually due to adenoma (benign tumor). Serum calcium usually ranges 12-20 mg/dl.
     2) **Ectopic cells as in some malignancy → ↑ PTH**
     3) **Excess intake of vitamin D:** or calcium or both. Usually it is due to over dosage or self-medication with vitamin D.
     4) **Milk-alkali syndrome:** This is hypercalcemia present in patients who received, for long periods, excessive absorbable alkalies and milk (source of calcium), for the treatment of peptic ulcer.
     5) **Bone diseases:** (↑ bone resorption) As in malignancy, leukemia, multiple myeloma and Paget`s disease.
     6) **Drugs:** As thiazide diuretics.
     7) **Other causes:** As thyrotoxicosis, Cushing's syndrome.
   - *b)* **Effects:**
     1) **Stone formation:** e.g renal stones.
     2) **Calcification** in different tissues.
2. **Hypocalcemia:**
   - *a)* **It is caused by:**
     1) Hypoparathyroidism.
     2) Alkalosis.
     3) Kidney diseases where activation of vitamin D is inhibited.
   - *b)* **Effects:**
     1) **Acute deficiency:** if ionized calcium is much decreased, tetany with carpopedal spasm results.
     2) **Chronic deficiency:** In children, Rickets and in adults, Osteomalcia.

II. *Phosphorus*

A. Sources:

1. Phosphate is present in all foods.
2. Milk and milk products.
3. Food additives.
4. Fish, meat, liver and kidney.
5. Leafy vegetables and egg yolk.

B. Absorption:

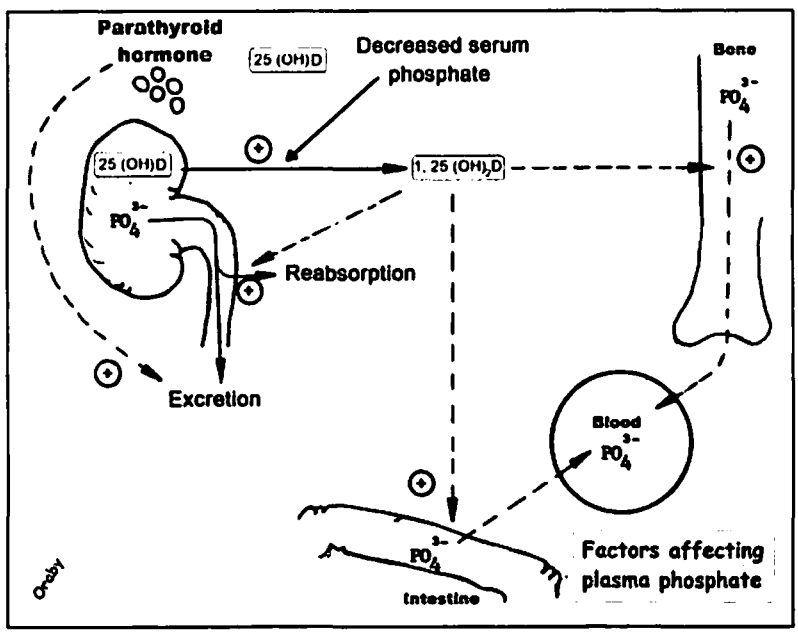
1. Phosphorus (in the form of phosphate) is absorbed by an active transport mechanism in the mid jejunum and enters blood stream via portal circulation.
2. Absorption is regulated by active vitamin D (calcitriol).
3. Factors affecting absorption of calcium will affect-in the same manner-the absorption of phosphorus.

C. Body phosphorus: Metabolism of phosphorus follows calcium inversely.

1. Total body phosphorus is about 800 g.
2. Most of phosphorus (80%) is present in the skeleton (bones and teeth) in the form of hydroxyapatite: $3\ Ca_3(PO_4)_2 .Ca(OH)_2$.
3. The other 20 % is present in other tissues **(mostly intracellular)** and body fluids.

D. Blood phosphorus:

1. **Normal** plasma inorganic phosphorus: 3-5 mg/dl.
2. Other forms are present:
   a) In plasma: phospholipids.
   b) In RBCs: organic phosphate e.g. ATP, glucose-6-phosphate.



3. **Factors affecting blood phosphorus:**
   a) **Parathyroid hormone (PHT):**
   PTH decreases blood phosphorus by stimulating its excretion (through inhibiting its renal tubular reabsorption).

b) **Active vitamin D "Calcitriol":**

1) Hypophosphatemia (↓ blood phosphate) stimulates directly the renal hydroxylation of $25(OH)D_3$ into $1,25(OH)_2D_3$ (calcitriol).

$$25\ (OH)\ D_3 \xrightarrow{\downarrow \text{Phosphate}} 1,25\ (OH)_2D_3$$

2) Calcitriol increases blood phosphorus through stimulation of:

i- Absorption of phosphorus from the intestine.

ii- Bone resorption i.e. mobilization of phosphorus from bones.

iii- Renal reabsorption by renal tubules.

E. <u>Functions of phosphorus</u>: (is the main intracellular anion). It enters in the structure of the following compounds:

1. **Bones and teeth** (in the form of hydroxyapatite).
2. **Plasma buffers** (phosphate buffers).
3. **Cellular components:**
   a) Nucleic acids: DNA, RNAs.
   b) Phospholipids: e.g. lecithin, cephalin.
   c) Phosphoproteins.
   d) Coenzymes: e.g. NAD, NADP.
   e) High energy phosphate compounds e.g. ATP, GTP, creatine and phosphate.
   f) Cyclic AMP and cyclic GMP.
   g) Carbohydrate intermediates e.g. glucose-6-phosphate, fructose-1-phosphate.

F. <u>Excretion</u>: Mostly (90%) is excreted in urine.

G. <u>Alterations of serum phosphate</u>:

1. **Causes of ↑ serum phosphate:**
   a) Hpyoparathyroidism.
   b) Acidosis.
   c) Hypervitaminosis D.
   d) RBCs Hemolysis.
2. **Causes of ↓ serum phosphate:**
   a) Hyperparathyroidism.
   b) Vitamin D deficiency.
   c) Renal tubular disease.
   d) Chronic alcoholism.

e) Excessive use of antacids.

f) Malabsorption.

H. **Requirements:** Same as for calcium.

III. *Magnesium*

A. **Sources:** Leafy green vegetables (containing chlorophyll).

B. **Absorption:** Occurs in the upper small intestine.

C. **Body magnesium:**

1. Mostly (70%) in the skeleton (bones and teeth).
2. The remaining 30% is present in the other tissues and body fluids mostly **intracellular.**

D. **Blood magnesium :**

1. Plasma magnesium: 2-3 mg/dl.
2. RBCs content of magnesium is 3 times greater than plasma content.

E. **Functions :**

1. It enters in the structure of skeleton (bones and teeth).
2. It activates many enzymes e.g. kinase enzymes.
3. It is required for the active transport of other cations (Ca^{++}, Na^{+}, K^{+}) across the cell membrane.
4. It is important for muscle contraction, nerve impulse transmission and it decreases neuromuscular excitability.

F. **Excretion:** Mostly (75 %) in feces.

G. **Requirements:** For adults: 400 mg/day.

IV. *Sodium:*

A. **Sources:** The main source is **table salt.**

B. **Absorption:** It occurs in small intestine (ileum). It is nearly completely absorbed.

C. Body sodium: It is regulated by aldosterone.

1. 2/3 of sodium is present in tissues and body fluids (sodium is the main extracellular cation).
2. About 1/3 of sodium is present in skeleton.

D. Plasma sodium: 137-143 mmol/L.

Factors affecting plasma sodium:

1. Aldosterone and the rennin angiotensin system (↑ Plasm sodium).
2. Changes in glomerular filtrate and renal blood flow.
3. Atrial natriuretic peptide.

E. Functions:

1. Maintenance of osmotic pressure and volume of plasma and extracellular fluid.
2. Transmission of nerve impulses.
3. Contraction of muscles.
4. Regulation of acid base balance.
5. **Sodium acts as substrate for Na^+/K^+ ATPase enzyme (sodium potassium pump).**

F. Excretion: Mainly (95%) in urine and sweat.

G. Requirements: For adults: 5 g/day.

H. Alterations of plasma sodium:

1. **Hypernatremia** (excess plasma sodium): It is caused by:
   a) **Cushing syndrome:** due to excessive glucocorticoids.
   b) **Conn`s disease:** due to excessive aldosterone secretion.
   c) **Diabetes insipidus (↓ADH):** due to rapid loss of water.
   d) **Drugs:** as ACTH or cortisone.
2. **Hyponatremia** (decrease plasma sodium) : It is caused by:
   a) **Addison`s disease:** due to deficiency of aldosterone.
   b) **Renal failure: where** renal reabsorption of sodium is inhibited.
   c) **Hypotonic dehydration:** where loss of water and sodium (electrolytes) is treated by administration of water only.
   d) **Diuretics:** e.g. thiazides, which block tubular reabsorption of sodium.

I. Toxicity: Hypertension in susceptible individuals.

V. *Potassium*

A. Sources: Vegetables, fruits and nuts.

B. Absorption: Rapidly occurs in the small intestine.

C. Body potassium:

It is regulated by aldosterone.

1. 2/3 of potassium is present in tissues and body fluids (potassium is the main intracellular cation).
2. About 1/3 is present in skeleton.

D. Plasma potassium: 3.5-5 mmol/L.

E. Functions:

1. Maintenance of **osmotic pressure** and volume of intracellular fluid.
2. Transmission of **nerve impulses.**
3. **Contraction of muscles.**
4. Regulation of **acid base balance.**
5. Substrate for **Na^+/K^+ ATPase.**

F. Excretion: Mainly in urine.

G. Requirements: 4 g/day.

H. Alterations of plasma potassium:

1. **Hyperkalemia** (excess plasma potassium): it is caused by:
   a) **Addison`s disease:** due to deficiency of aldosterone.
   b) **Acidosis:** (respiratory or metabolic): due to shift of K^+ from intra to extracellular in exchange with H^+.
   c) **Tissue necrosis:** e.g. major trauma and burns due to leakage of tissue contents of potassium.
   d) **Acute renal failure and advanced chronic renal failure,** associated with oliguria.
   e) **Uncontrolled diabetes mellitus:** the lack of insulin and associated acidosis prevents K^+ from entering cells.
   - **Acute hyperkalemia :** if plasma K^+ gets more than 6.5 mmol/L, cardiac arrhythmias and even cardiac arrest may result .

2. **Hypokalemia:** (decreased plasma potassium): it is caused by:
   a) **Alkalosis:** (respiratory or metabolic).
   b) **Treatment of hyperglycemia:** by insulin without giving potassium because insulin helps K^+ to enter cells.
   c) **Excessive vomiting and diarrhea.**
   d) **Cushing syndrome:** due to excessive glucocorticoids.
   e) **Primary and secondary hyper-aldosteronism.**
   f) **Diuretic therapy.**

VI. *Chloride*

A. Sources: Table salt.

B. Absorption: Occurs in small intestine.

C. Plasma chloride: 96-106 mmol/L.

D. Functions:

1. Chloride is the **main extracellular anion.** Together with sodium, it maintains the osmotic pressure and volume of plasma and extracellular fluid.
2. Chloride ions are essential for formation of HCl in the stomach.
3. Activation of enzymes: Cl^- activates salivary and pancreatic amylase enzymes.

E. Excretion: Mainly in urine.

F. Requirements: For adults: 5 g/day.

G. Alterations of plasma chloride:

1. **Hyperchloremia:**
   a) Hyperchloremic acidosis:
   Occurs when HCO_3^- is lost in exchange with chloride as in renal tubular acidosis and hyperventilation.
   b) Glomerulonephritis.
   c) Eclampsia (toxicity of pregnancy).

2. **Hypochloremia:**
   a) **Hypochloremic alkalosis:** decreased plasma chloride due to:
      1) Intestinal obstruction → excessive vomiting.

2) This leads to decrease plasma chloride and increase plasma bicarbonate as compensatory mechanism, causing alkalosis.

b) Addison's disease.

c) Diabetes insipidus.

Microminerals (Trace Elements)

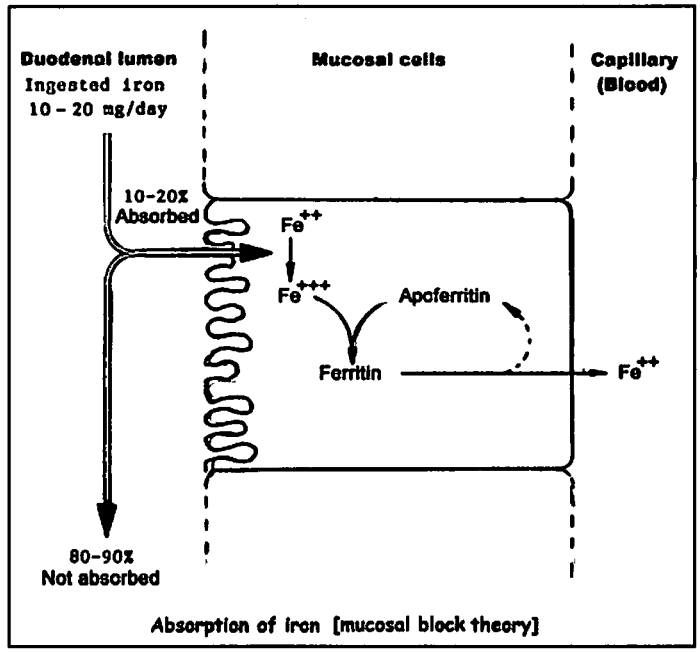
I. *Iron*

A. Sources:

1. Liver, heart, kidney, spleen and fish.
2. Sugar cane syrup (molasses).
3. Dates and egg yolk.
4. Contrary to popular belief, spinach is a poor source of iron because it is bound to phytate, which is difficult to absorb.

B. Absorption: Absorption of iron occurs in the duodenum and the proximal part of the jejunum.

1. Diet contains about 10-20 mg iron/day. Usually only 10-20% of this amount is absorbed.
2. **Mechanism: Mucosal block theory:**



Absorption of iron [mucosal block theory]

a) According to this theory, iron is absorbed in the ferrous state (Fe^{++}). Inside mucosal cells, it is oxidized to ferric state (Fe^{+++}) and combines with apoferritin to form ferritin.

b) Ferritin liberates ferrous ions into the capillaries (plasma) and apoferritin is regenerated again. The rate of this liberation depends on body needs.

c) The intestinal content of apoferritin is limited and when all apoferritin molecules become saturated with iron, absorption is blocked.

3. **Factors affecting iron absorption:** most of dietary iron is present in the ferric state (Fe^{+++}) as ferric organic compounds.

   a) **Factors promoting iron absorption:**

      1) **Cooking of food and gastric HCl** facilitates the liberation of ferric ions (Fe^{+++}) from organic compounds.

      2) **Reducing substances:** vitamin C and cysteine (-SH) of dietary protein help the reduction of ferric ions (Fe^{+++}) into the absorbable ferrous (Fe^{++}) state.

      3) **Body needs:** absorption occurs only if the body is in need to iron. More iron is absorbed when there is iron deficiency or when erythropoiesis is increased.

   b) **Factors inhibiting iron absorption:**

      1) **High dietary phosphate and phytate:** They form insoluble, non-absorbable organic iron complexes.

      2) **Steatorrhea:** Where fatty acids form non-absorbable iron soaps.

      3) Alkalies and tea.

4. **Recent theory for iron absorption:**

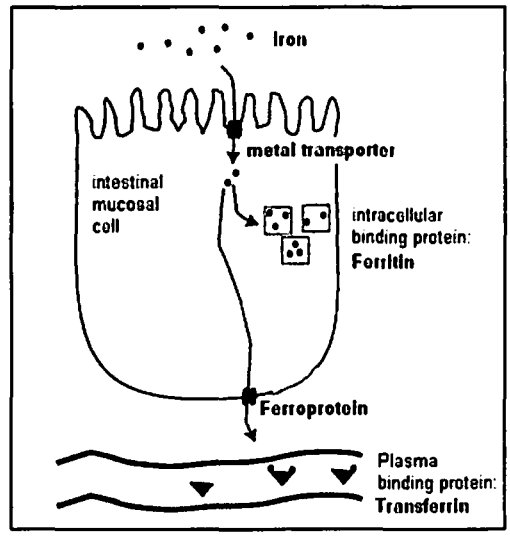
   a) On the brush borders of mucosal cells, iron binds with specific carrier protein called metal transporter.

   b) It passes inside intestinal mucosal cells.

   c) Inside mucosal cells, iron either:

      1) leave the intestinal cell and enter the plasma via the **transporter** known as **ferroportin**.

      2) Incorporated into ferritin formation i.e. ferritin acts as storage compound and not as a carrier for iron absorption.



C. Body iron:

1. The total body iron of an adult male is 3-5 grams.
2. It is distributed as follows: RBCs iron (Hemoglobin): 66%, tissue iron (33%) and plasma iron 1%.
3. **RBCs iron:** (hemoglobin): see hemoglobin metabolism.
4. **Tissue iron:** it includes:

a) **Available iron forms (29 %):** i.e. can be used by tissues when there is body need.

1) **Ferritin:**

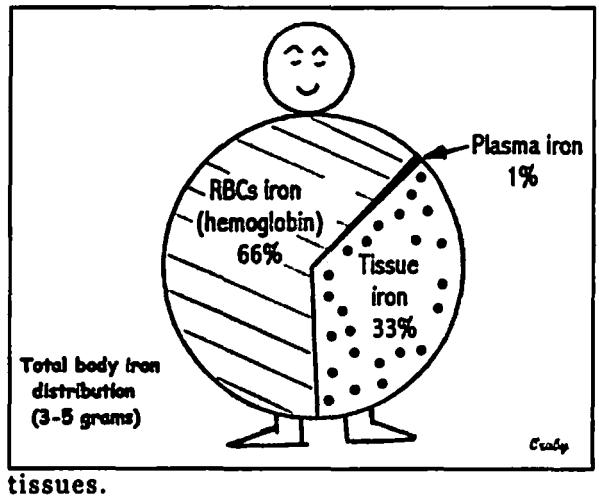
i- It is the main storage form of iron.

ii- It is formed of a protein called apoferritin, which can carry 3500-4000 atoms of iron to form ferritin.

iii- It is present in iron stores: liver, spleen, bone marrow and intestine.

2) **Hemosedrin:**

i- When body contains very high content of iron more than the capacity of apoferritin, some of iron is found in granules called hemosedrin that deposited in tissues.



ii- These granules are composed of iron, protein, and polysaccharides.

iii- Hemosedrin may be a degraded ferritin.

b) **Non-available iron forms (4%):** cannot be used even if there is body needs. All these forms are **hemoproteins** i.e. contain heme ring.

1) **Myoglobin:**

i- It is hemoprotein formed of a single heme ring attached to one long polypeptide chain.

ii- It is present in muscles **and heart.**

iii- It acts as oxygen reservoir for quick utilization by contracting muscles.

2) **Respiratory cytochromes (b, c_1, c, a, a_3):**

i- These are components of respiratory chain in mitochondria.

ii- They act as electron carriers.

3) **Catalase and peroxidase:**
   i- These are two enzymes that act on the toxic hydrogen peroxide (H_2O_2) converting it into H_2O.

4) **Tryptophan oxygenase (pyrrolase):**
   i- This enzyme is important for tryptophan metabolism.

5) **Cytochrome P_{450}:**
   i- These are a specific group of enzymes that present in liver, lung, kidney, gut, adrenal cortex, heart, and brain. They are used in xenobiotics metabolism.

5. **Plasma iron:**

a) **Plasma iron: Ranges from 60 - 160 ug/dl.**

b) **Plasma transferrin:**

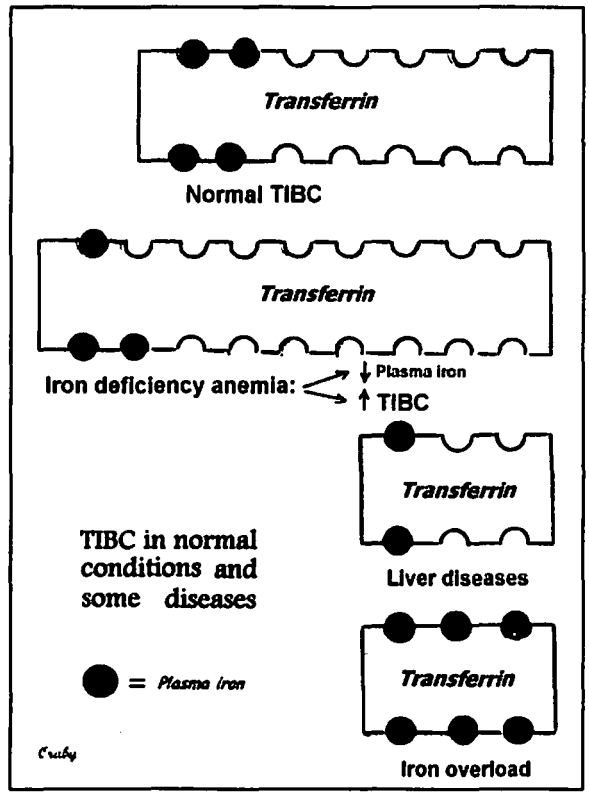
1) **This is a plasma glycoprotein that acts as carrier for iron. It is synthesized in the liver.**

2) Each molecule can carry 2 atoms of iron in ferric state (Fe^{3+}).

3) Transferrin may carry up to **180-450 ug iron/dl**. This is known as total iron binding capacity of transferrin (TIBC). As the plasma iron is 60-160 ug/dl, thus only 30% of the TIBC of transferrin is saturated.

4) TIBC is therefore defined as ***maximum amount of iron that can be carried by transferrin per deciliter.***

5) **Abnormalities of plasma TIBC concentration:**
   i- **In iron deficiency anemia:** Plasma iron is decreased. Liver synthesizes more transferrin with subsequent increase of TIBC.
   ii- **In liver diseases:** Both plasma iron and transferrin synthesis tend to decrease (↓ plasma iron and ↓TIBC).
   iii- **In iron overload:** transferrin synthesis is inhibited. This leads to increased plasma iron and decreased Total iron binding capacity.



c) **Plasma ferritin:**

1) Ferritin is present mainly in iron stores: liver, spleen, bone marrow and intestine.
2) **Very low concentration** of ferritin is present in plasma.
3) Measurement of plasma ferritin gives a good idea about body iron stores.
   - i- **A low plasma ferritin** indicates the presence of depleted iron stores e.g. in iron deficiency anemia.
   - ii- **A raised plasma ferritin** is found in iron overload and also in many patients with liver disease and cancer.

D. <u>Functions of iron</u>: Iron enters in the structure of the following compounds:

1. Hemoglobin: which carries oxygen.
2. Myoglobin: which stores oxygen.
3. Respiratory enzymes: which use oxygen.
4. Cytochrome P_{450}: which detoxicates drugs and oxygen.
5. Other enzymes: catalase, peroxidase and tryptophan oxygenase.

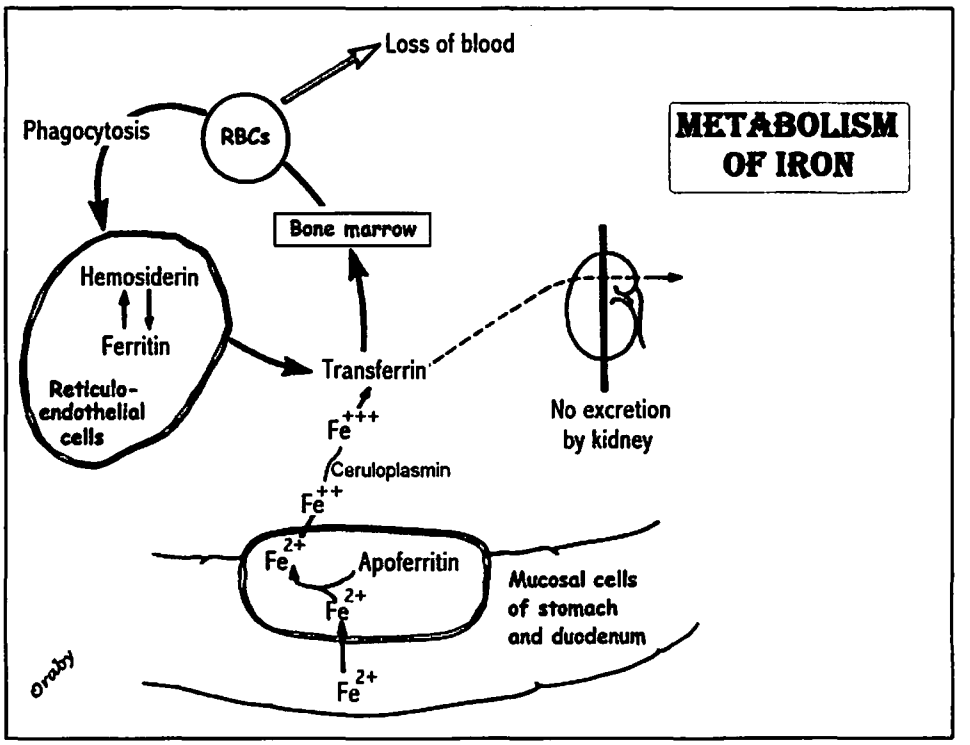
E. <u>Transport and storage of plasma iron :</u>

1. Absorbed iron enters in the portal blood in ferrous state (Fe^{++}).

2. In the plasma, it is rapidly oxidized to ferric state (Fe^{+++}). A protein containing copper called ceruloplasmin catalyzes this oxidation.

$$Fe^{++} \xrightarrow{\text{Ceruloplasmin}} Fe^{+++}$$

3. Then ferric ions are carried by a transferrin, which is taken mostly by bone marrow to synthesize hemoglobin.
4. Iron, from iron stores (ferritin) can be released into plasma and carried by transferrin to be utilized by bone marrow and other tissues.



F. Excretion:

1. Iron excreted in the feces is mainly exogenous i.e. dietary iron that has not been absorbed.
2. In males there is an average loss of endogenous iron of about **1 mg/day.** It is derived from desquamated cells from skin and the intestinal mucosa.
3. In females, there are additional sources of loss, due to menstruation and pregnancy.
4. Urine contains negligible amount of iron.

G. Requirements:

1. **Adults:** 10 mg/day.
2. **Pregnant and lactating women:** 30 mg/day.

H. Alterations of plasma iron:

1. **Iron deficiency anemia:**
   - *a)* **Causes:**
     1) Deficient intake.
     2) Impaired absorption: e.g. steatorrhea, abdominal surgery.
     3) Excessive loss e.g. menstrual loss, gastrointestinal bleeding due to some parasites (anchylostoma).
   - *b)* **Biochemical changes:**
     1) Plasma iron is decreased.
     2) Plasma TIBC is increased.
     3) Plasma ferritin is decreased.
     4) RBCs show: hypochromic, microcytic cells.
2. **Iron overload:**
   - *a)* **Causes:**
     1) Repeated blood transfusion.
     2) Intravenous administration of iron.
     3) **Hemochromatosis** (hemosiderosis, bronze diabetes):
        - i- This is a rare hereditary disease characterized by abnormal increase of iron absorption.
        - ii- Iron is deposited in the form of hemosedrin in:
          - **Liver:** causing liver cirrhosis.
          - **Pancreas:** causing fibrosis and diabetes mellitus.
          - **Skin:** causing bronze discoloration of skin.
   - *b)* **Biochemical changes:**
     1) Plasma iron is increased.
     2) Plasma TIBC is decreased.
     3) Plasma ferritin is increased.

II. *Copper*

A. Sources: The richest sources are: liver, kidney, dried legumes and nuts.

B. Absorption: Mainly occurs in the upper small intestine.

C. Body copper:

1. The adult human body contains about 100-150 mg of copper.
2. 64 mg (50%) are found in muscles and the remaining present in other tissues including liver and bones.

D. Blood copper:

1. **In the plasma: 90 ug/dl.** It is present in association with 2 proteins:
   a) **Ceruloplasmin: (90%)** A copper binding protein. Each molecule can bind 6 atoms of copper. It acts as ferroxidase enzyme during iron metabolism ($Fe^{++} \rightarrow Fe^{+++}$).
   b) **Albumin: (10%)** It is loosely bound form of copper. It acts as a carrier for transport of copper in plasma.
2. **In red cells: 100 ug/dl.** It is present in association with the enzyme **superoxide dismutase** (erythrocuprein), which deals with the toxic free radical superoxide ion (O_2^-) generated during aerobic metabolism.

E. Functions:

1. **Copper is essential for:**
   a) Hemoglobin synthesis.
   b) Bone formation.
   c) Maintenance of myelin of the nerves.
2. **Copper is essential constituent of several metalo-enzymes:**
   a) **Ceruloplasmin:** which oxidizes Fe^{++} into Fe^{+++} in the plasma.
   b) **Superoxide dismutase:** which eliminates the toxic effect of superoxide ions (O_2^-).
      - Superoxide dismutase is present in RBCs (erythrocuprein) , liver (hepatocuprein) and brain (cerebrocuprein).
   c) **Cytochrome oxidase** (see respiratory chain, part II).
3. **Copper activates many enzymes:** e.g. tyrosinase, uricase and dopamine hydroxylase.

F. Excretion:

1. Mainly with bile.
2. Urinary excretion is minimal due to large molecular weight of ceruloplasmin.

G. Requirements: Adults: 2 - 3 mg/day.

H. Alterations of plasma copper:

1. **Hypercupermia:** (excess plasma copper and ceruloplasmin): Ceruloplasmin is considered as acute phase protein i.e. its plasma level is increased in infections and malignancy.

2. **Hypocupermia:** (decreased plasma copper and ceruloplasmin):
   a) **Anemia:** Hypochromic and microcytic anemia.
   b) **Impaired bone mineralization.**
   c) **Wilson`s disease (hepatolenticular degeneration):**
      1) This disease is characterized by accumulation of large amounts of copper in:
         i- **Liver** causing hepatic cirrhosis.
         ii- **Lenticular nucleus of the brain** causing lenticular degeneration with abnormal movement.
         iii- **Cornea:** Causing greenish-brown discoloration of the corneal margin, which is called: **Kayser - Fleisher rings.**
         iv- **Kidney** causing renal tubular damage which leads to:
            - Increased excretion of copper and ceruloplasmin. This results in low serum copper (hypocupremia) and ceruloplasmin.
            - Increased excretion of amino acids. This results in aminoaciduria.
      2) **The cause of the disease** is most probably due to either:
         i- Excessive copper absorption from intestine.
         ii- Inadequate excretion of copper in bile.

III. Zinc

A. Sources: Meat, liver, eggs, seafood, milk, and whole grain cereals.

B. Absorption: Zinc absorption occurs mainly in small intestine, especially from the duodenum.

C. Body zinc:

1. The adult male body contains about 2 g of zinc.
2. About 20 % of total body zinc is present in the skin.
3. The remaining is present in skeleton (bones and teeth), spermatozoa, prostate, epididymis and pancreas.

D. Plasma zinc: Adults: 70-150 ug/dl.

E. Functions of zinc:

1. Zinc is essential for growth and reproduction.
2. It plays a role in tissue repair and wound healing.
3. Zinc forms a complex with insulin in β islet cells of the pancreas. This helps crystallization, storage and release of insulin.
4. Zinc is required for mobilization of vitamin A from the liver and subsequently maintains the normal concentration of vitamin A in plasma.
5. Zinc is essential component of a number of enzymes e.g.:
   a) Alkaline phosphatase.
   b) Carbonic anhydrase.
   c) Superoxide dismutase
   d) Carboxypeptidase.
   e) RNA polymerase.

F. Requirements: An adult male: 10-20 mg/day.

G. Excretion: Mainly in feces (mostly unabsorbed dietary zinc).

H. Zinc deficiency: It causes:

1. Hypogonadism.
2. Poor healing of wounds.
3. Poor appetite and retard growth in children.
4. Liver cirrhosis.
5. Diarrhea and dermatitis.
6. Confusion, apathy and depression.

IV. *Iodine*

A. Sources:

1. Iodinized table salt will provide daily body needs.
2. Fish, seafoods, weeds, and vegetables grown near seaboard.

B. Absorption: Occurs mainly from small intestine.

C. Body iodine:

1. The adult male body contains about 25-50 mg iodine.
2. It is present in:
   a) Thyroid gland: (50%): as thyroglobulin.
   b) Other tissues and body fluids (50%): as T_3 and T_4.

D. Plasma iodine:

1. Organic iodine: 4-8 ug/dl.
2. Inorganic iodine: 1-2 ug/dl.

E. Functions:

The only known function of iodine is the formation of thyroid hormones (T_3 - T_4).

F. Excretion: Mainly (70%) in urine.

G. Requirements:

For adult: 100 - 150 ug/day.

H. Deficiency:

Hypothyroidism (myxodema in adults and cretinism in children).

V. *Selenium*

A. Selenium is an antioxidant. It is an essential component of the enzyme glutathione peroxidase (GSH-Px) which catalyzes the reaction:

$$2\ GSH + H_2O_2 \xrightarrow{GSH\text{-}Px} GSSG + 2\ H_2O$$

B. This reaction acts as protective mechanism against the oxidative damage of hydrogen peroxide (H_2O_2) and fatty acid hydroperoxide by destroying them:

1. In RBCs, it protects hemoglobin and red cell membranes.
2. In liver, it is important for detoxifying lipid hydroperoxides.
3. In lens of the eye, it prevents its oxidative damage.

C. Deficiency of selenium (GSH-Px): It causes:

1. Hemolytic anemia.
2. Liver cirrhosis.
3. Cataract.

VI. *Manganese*

A. Manganese is essential for:

1. Normal bone structure.
2. Reproduction (spermatogenesis and ovulation).
3. Normal function of the central nervous system.

B. Manganese is a component of:

1. Pyruvate carboxylase enzyme.
2. Superoxide dismutase enzyme.

C. Manganese activates: the arginase enzyme.

VII. *Cobalt*

A. Functions:

1. Cobalt is a component of vitamin B_{12}, which is necessary for normal blood cell formation. Cobalt gives vitamin B_{12} **its red color.**
2. **Enzymes requiring vitamin B_{12}** for their activities are:
   a) Methylmalonyl CoA mutase.
   b) Methyltetrahydrofolate oxidoreductase.
   c) Homocysteine methyltransferase.
   d) Ribonucleotide reductase.

B. Deficiency of cobalt ➔ ↓ vitamin B_{12} causes **pernicious anemia** (for details see vitamin B_{12}, part I).

VIII. *Chromium*

- It acts only together with insulin to promote glucose utilization.
- Its deficiency leads to impairment of glucose utilization by tissues.

IX. *Molybedenum*

- It is a component of oxidase enzymes e.g. xanthine oxidase.

X. *Flouride*

- It increases the hardness of bones and teeth.
- Its deficiency causes **dental carries** and osteoporosis.
- It is supplied in drinking water to support bones and teeth.
- Excess flouride leads to **flourosis**: mottling and discoloration of the enamel of teeth and changes in bones.

Water metabolism

I. Water is the most abundant compound in living cells, which usually constitutes about 60% of adult body weight.

A. Functions of water:

1. Water is a **solvent** for many ionic compounds and neutral molecules.
2. Water is important to **maintain the structure and functions of macromolecules of cells** e.g. proteins and polynucleotides.
3. **Regulation of body temperature** by evaporating the moisture in the lungs and from the skin.
4. Water is the main constituent of all **body fluids.**

B. Total body water:

Total body water is distributed between 2 main compartments:

1. **Intracellular fluid:** This is the fluid within body cells. It constitutes about **40 %** of total body weight, and **2/3** of total body water.
2. **Extracellular fluids:** This is all the fluids outside the body cells. It constitutes about **20 %** of total body weight and **1/3** of total body water. Extracellular fluid can be subdivided into:
   a) **Plasma.**
   b) **Interstitial and lymph fluid.**
   c) **Transcellular fluids:** These are fluids present inside organs as liver, salivary glands, and mucous membranes of the respiratory and gastrointestinal tract. It also includes fluids in spaces within the eye, CSF and other body fluids.
   d) **Bones, dense connective tissue and cartilage:** because of relative avascularity, these tissues do not exchange fluid or electrolytes with plasma and classified as subdivision of extra cellular fluid.

II. *Water balance*: It means that water intake is equal to water loss.

Water intake: Water is derived from:	
1. **Drinking water and other liquids**	**1400 ml/day**
2. **Solid foods** (approximately, 80% of normal diet consists of water)	**800 ml/day**
3. **Metabolic water** (derived from oxidation of organic food inside the cells).	**300 ml/day**
Total:	**2500 ml/day**
Water loss: water is lost from the body through 4 routes:	
1. **Skin:** as sensible (sweat) and insensible perspiration.	**850 ml/day**
2. **Lungs:** as water vapor in the expiration	
3. **Kidneys** :as urine	**1500 ml/day**
4. **Intestine** :as feces	**150 ml/day**
Total:	**2500 ml/day**

A. <u>In very hot weather</u>: or during periods of prolonged heavy exercise, water loss in sweat may increase up to 3000 ml / hour, which could deplete the body water rapidly.

B. <u>Additional water losses in diseases</u>:

1. **In kidney diseases**, in which concentrating ability is limited.
2. **Diarrhea and vomiting** especially in infants.
3. **Fevers** due to excessive sweating.
4. **Individuals** subjected to high environmental temperature.

C. <u>Disturbance of water balance</u>: In spite of large amount of water content (60% of adult body weight), there is no water reserve in the body. Loss of 5% of body water making the subject strongly thirst, loss of 10% making him very ill and he will die if loses 20% of total body water.

D. <u>Dehydration:</u> is the increase of water loss compared to water intake. It may be due to pure water loss or electrolyte deficit:

1. **Pure water loss: (Hypertonic dehydration):**

   a) **Causes:**

   Restriction of water supply for any reason, or when the losses are excessive.

   b) **Metabolic disturbances:**

   The water loss exceeds the rate of electrolyte loss. The extracellular fluid becomes concentrated and hypertonic to the cells. Water then shifts from the cells to the extracellular

space to compensate, creating what is called: **intracellular dehydration.**

c) **Symptoms:**

Include severe thirst, nausea and vomiting, hot and dry body, dry tongue and loss of coordination and concentrated urine of small volume.

d) **Treatment:**

Intracellular dehydration is corrected by giving water by mouth or intravenously until symptoms disappear and the urine volume is restored.

2. **Electrolytes loss (deficit): (Hypotonic dehydration):**

a) **Causes:**

This occurs in conditions like sunstroke when water and electrolyte loss is corrected by giving water only. This will lead to a deficiency of electrolytes in the presence of normal or excess total body water.

b) **Metabolic disturbances:**

The deficiency of sodium in extracellular fluid results in hypotonicity of this fluid compartment so, some water passes into the cells, which are hypertonic to the extracellular fluid, causing the so-called: **intracellular edema.** This will lead to diminution in extracellular fluid volume with decrease in blood volume; fall in blood pressure, and consequent impairment in renal function.

c) **Symptoms:**

The patient becomes progressively weaker, but he does not complain of thirst and his urine volume is not markedly changed.

d) **Treatment:**

Extracellular dehydration is corrected by giving water and electrolytes (saline) intravenously.

Chapter 6 *Acid base regulation (Balance)*

I. *Introduction:*

A. The most important factor, which must be considered in regulation of acid base balance, is the **hydrogen ion concentration.**

B. **Definitions:**

1. **An acid:** it is a donor of hydrogen ions (protons, H^+) and it lowers the pH e.g. hydrochloric acid, carbonic acid.
2. **A base:** it is an acceptor of hydrogen ions (protons, H^+) and it raises the pH e.g. sodium hydroxide, sodium bicarbonate.
3. **pH:** it is the negative logarithm of hydrogen ion concentration:

$$pH = -\log (H^+)$$

4. **Blood pH:**
   a) Blood pH is normally kept within the range 7.37 to 7.43.
   b) The arterial blood (pH = 7.4) is slightly more alkaline than venous blood (pH = 7.37).

C. **Effect of food metabolism on pH:**

1. The metabolic processes that occur to the consumed mixed diet cause an overall production of acids. e.g. lactic acid from RBCs and muscles during exercise, phosphoric acid from metabolism of sulfur containing amino acids .
2. The pH of the extracellular fluids including blood is kept constant (7.4 ± 0.03) in spite of the large amount of acids (H^+), which are continuously added to the blood.
3. The removal of excess H^+ can be achieved by blood buffers.

II. *Acid base balance:*

It is the equilibrium between the acid production and the rate of its removal.

A. **Buffer and blood buffers:**

1. **Buffer:**
   a) **Buffer** is a solution, which resists the change in pH when an acid or alkali is added to it.
   b) **Buffers are usually consisted of:**
      1) Mixture of a weak acid and its salt with strong base.

2) Or mixture of a weak base and its salt with strong acid e.g. carbonic acid (H_2CO_3) / sodium bicarbonate ($NaHCO_3$).
   - Weak acid = carbonic acid (H_2CO_3).
   - Salt of weak acid = sodium bicarbonate ($NaHCO_3$).
   - Strong base = sodium. [***Note: Sodium** by itself does not affect pH, but it can be **considered as a base** because in the presence of hydroxyl ions (OH^-) it can form the strongly ionized base, sodium hydroxide].*

2. **Blood buffers:** Blood buffers are either plasma (extracellular fluids) buffers or RBCs buffers:

a) **Plasma buffers**

1) **Types:**
   - i- Carbonic acid/bicarbonate buffer: ($H_2CO_3/NaHCO_3$). It is called **alkali reserve**
   - ii- Phosphate buffer: Sodium acid phosphate/Sodium alkaline phosphate (NaH_2PO_4 /$NaHPO_4$).
   - iii- Plasma protein buffer: (H protein /Na proteinate).

2) **Plasma buffers** are concerned with neutralization of non-volatile acids e.g. lactic, phosphoric acids. **Buffers of RBCs** are considered with neutralization and carriage of CO_2.

3) **In plasma, the carbonic acid / bicarbonate** buffer is the most important buffering mechanism. **In RBCs, hemoglobin plays** a major part in the total buffering power of the blood.

b) **RBCs buffers** are:

1) Hemoglobin buffer: Acid reduced hemoglobin / potassium hemoglobinate (HHb/KHb).
2) Oxyhemoglobin buffer: ($HHbO_2$ / $KHbO_2$).
3) Carbonic acid / bicarbonate buffer: (H_2CO_3 / $NaHCO_3$).

B. The Henderson - Hasselbalch equation:

This equation represents the relationship between pH and pK (acid dissociation constant) of a weak acid. A weak acid as carbonic acid (H_2CO_3): ionizes as follows:

$$H_2CO_3 \leftrightarrows H^+ + HCO_3^-$$

a. According to the law of mass action:

$$\frac{[H^+] \times [HCO_3^-]}{[H_2CO_3]} = K$$

Where K is the dissociation constant of carbonic acid

$$\therefore [H^+] \times [HCO_3^-] = K\ [H_2CO_3]$$

b. By dividing both sides by $[HCO_3^-]$

c. By taking the log of both sides:

$$\therefore \text{Log } [H^+] = \text{Log } \left(\frac{K \times [H_2CO_3]}{[HCO_3^-]}\right)$$

$$\therefore \text{Log } [H^+] = \text{Log } K + \text{Log } \frac{H_2CO_3}{[HCO_3^-]}$$

d- By multiplying both sides by -1

$$\therefore - \log [H^+] = - \log K - \log \frac{[H_2CO_3]}{[HCO_3^-]}$$

$$\therefore - \log [H^+] = pH$$

$$\therefore - \log K = pK$$

$$\therefore pH = pK - \log \frac{[H_2CO_3]}{[HCO_3^-]}$$

e- Then to remove the minus sign, invert the last term :

$$pH = pK + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

f- $\therefore$ Normal pH of blood is 7.4 and $\therefore$ pK of carbonic acid is 6.1

$$\therefore 7.4 = 6.1 + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

$$7.4 - 6.1 = \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

$$\therefore 1.3 = \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

Antilog 1.3 = 20

Therefore $\frac{20}{1} = \frac{HCO_3^-}{H_2CO_3}$

The plasma carbonic acid (H_2CO_3) is in equilibrium with the dissolved carbon dioxide (CO_2):

$$\therefore H_2CO_3 \leftrightarrow H_2O + CO_2$$

Thus it can be concluded that any change in dissolved (respiratory) carbon dioxide or (metabolic) bicarbonate will disturb the normal ratio 20/1 and causes change in blood pH.

III. *Transport and buffering of CO_2 in blood:*

A. Hemoglobin and oxyhemoglobin are the most important blood buffers present in RBCs.

B. <u>At the tissues</u>:

1. CO_2 produced in the course of metabolism enters the blood (RBCs) which is hydrated in the presence of carbonic anhydrase enzyme to form carbonic acid (H_2CO_3).
2. H_2CO_3 is then dissociated to form H^+ and bicarbonate (HCO_3^-).

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3 \rightarrow H^+ + HCO_3^-$$

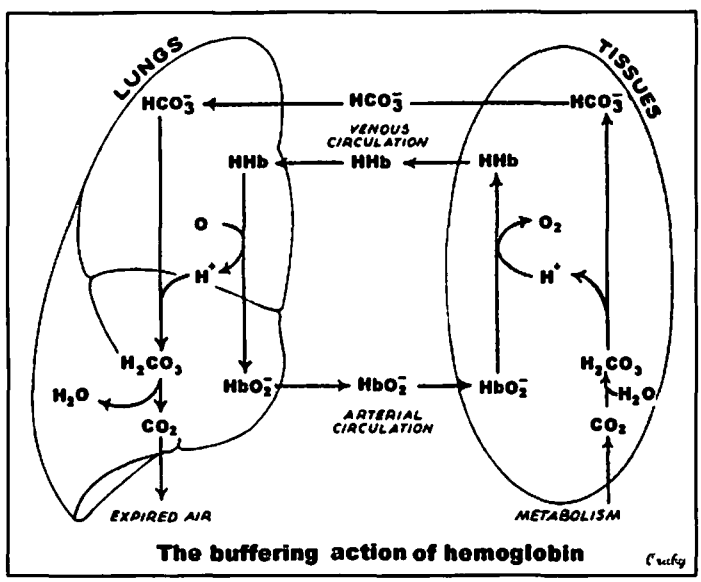
3. At the same time oxyhemoglobin dissociates and delivers oxygen to the cells and reduced hemoglobin is formed. This process is helped by low oxygen tension in the tissues as well as the effect of increased CO_2 tension, Bohr effect, and 2,3 BPG (see hemoglobin metabolism).
4. Reduced hemoglobin acts as an anion acceptor, forming the so called acid-reduced hemoglobin (HHb) which is very weak acid .

C. <u>At the lungs</u>:

1. The formation of oxyhemoglobin from reduced hemoglobin must therefore release hydrogen ions (H^+).
2. H^+ will react with bicarbonate to form carbonic acid ($H_2CO_3^-$).
3. Because of the low CO_2 tension in the lungs, the equilibrium then shifts toward the production of CO_2 which is continuously eliminated in the expired air:

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$$

D. As discussed above, very little change in pH occurs because the H^+ released at the tissues are buffered by reduced hemoglobin, and released at the lungs, which reacts with (HCO_3^-) to form H_2O and CO_2.



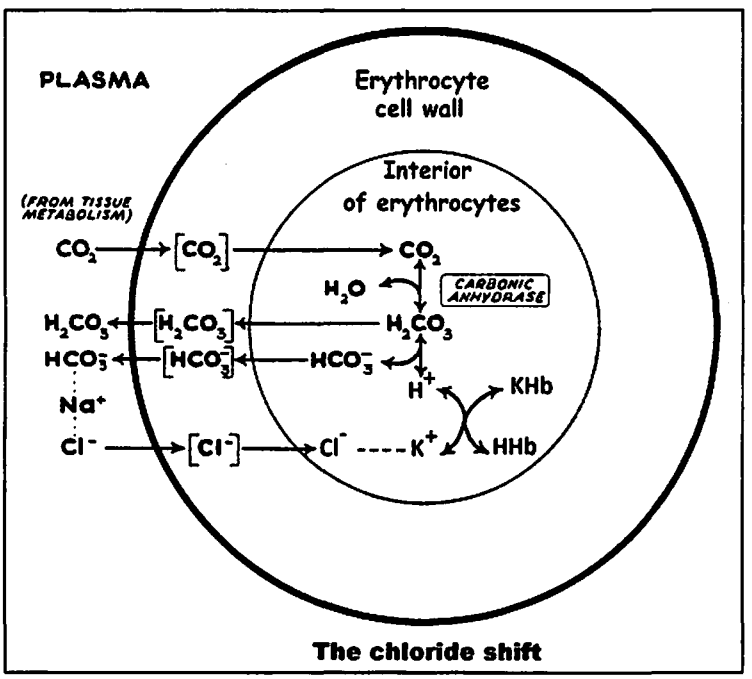
The buffering action of hemoglobin

E. Chloride shift:

1. As mentioned before, CO_2 reacts with water to form carbonic acid mainly inside the red cells, a reaction catalyzed by carbonic anhydrase enzyme.
2. A little of carbonic acid returns to the plasma and the remainder is buffered by hemoglobin (KHb):

$$KHb + H_2CO_3 \rightarrow HHb + K^+ + HCO_3^-$$

3. The red cell membrane is permeable to bicarbonate ions, but impermeable to potassium (K^+) ions.
4. Bicarbonate ions diffuse outside the RBCs in exchange for chloride ions which shifts into the cell in order to maintain electrical neutrality across the erythrocyte membrane. Chloride ions are neutralized by potassium while sodium bicarbonate is formed in the plasma.
5. This process occurs when CO_2 tensions are increased and this explains the higher chloride content in venous RBCs than arterial RBCs.
6. In arteries, where CO_2 tension is reduced, the reverse occurs i.e. the chloride leaves the cells and enters the plasma.

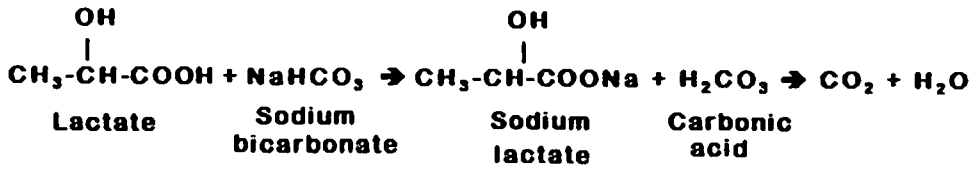


The chloride shift

IV. *Buffering of non volatile acids:*

A. As mentioned before, the non volatile acids produced by various metabolic pathways can be buffered by 3 types of plasma buffers, the most important one is carbonic acid / bicarbonate (H_2CO_3 / $NaHCO_3$).

B. Example: Lactic acid produced from RBCs and during muscle exercise is buffered as follows:



C. Thus the relative strong lactic acid is transformed to a weak carbonic acid (H_2CO_3) which dissociates at lungs to H_2O and CO_2.

D. CO_2 is then removed in the expired air.

Disturbance of acid - base balance: (Acidosis and alkalosis):

I. Introduction:

A. The normal ratio of bicarbonate / carbonic acid is 20/1.

B. Any alteration in the concentration of one of 2 buffer components is immediately compensated by alteration in the other to maintain the ratio constant (20/1).

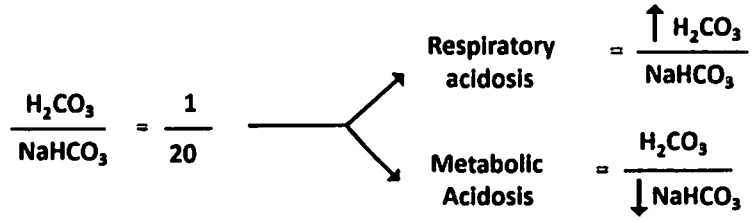
C. Disturbance in that ratio will lead to change in pH, which results in either acidosis or alkalosis.

II. Acidosis:

A. **Definition:** Acidosis is a state of increase hydrogen ion concentration of the blood i.e. the blood pH is lowered than normal (less than 7.37).

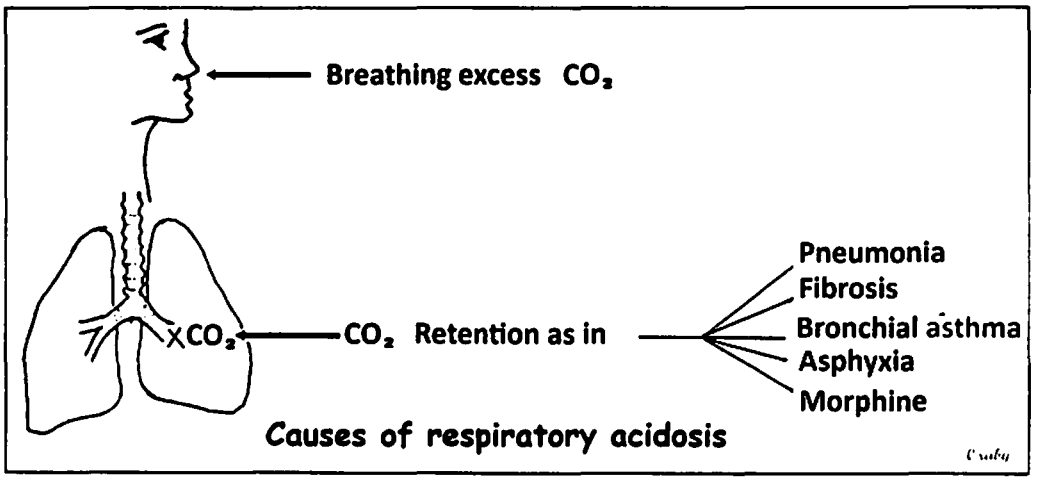
B. **Types of acidosis:**

1. **Respiratory acidosis:** Due to a primary increase in the concentration of carbonic acid content (CO_2 tension) resulting from decreased ventilation.
2. **Metabolic acidosis:** Due to a primary decrease in the concentration of bicarbonate ($NaHCO_3$), resulting from abnormal metabolism.



C. <u>Respiratory acidosis</u>: due to increased carbonic acid content of the blood (↑ CO_2 tension).

1. **Causes:**
   a) Breathing air containing abnormally high percentage of CO_2.
   b) CO_2 retention in the lungs as in:
      1) Pneumonia, pulmonary fibrosis, bronchial asthma and pulmonary emphysema.
      2) Mechanical asphyxia.
      3) Morphine poisoning: which depresses respiratory center.
      4) Cardiac decomposition: Due to pulmonary congestion and slowed circulation.
      5) Disorders in the C.N.S on the respiratory muscles or extreme obesity.



Causes of respiratory acidosis

2. **Compensatory mechanism:**
   a) The condition starts with a fall in pH and it is called: uncompensated respiratory acidosis.
   b) Then the kidneys respond by reabsorption of more bicarbonate ($NaHCO_3$) to restore the ratio 20/1, and the condition is called compensated respiratory acidosis.
   c) There is increase in urinary secretion of H^+, NH_4^+ and Cl^- to help reabsorption of HCO_3^-.
3. **Serum bicarbonate:** Serum bicarbonate is increased in the compensated respiratory acidosis i.e. alkali reserve is increased.

D. <u>Metabolic acidosis</u>: (due to decreased bicarbonate content of the blood).

1. **Causes:**
   a) **Overproduction of acids as in:**
      1) **Diabetes mellitus:** due to ketosis and dehydration.

2) **Ketosis:** occurs in diabetes mellitus, starvation and carbohydrate poor diet.

3) **Lactic acidosis:**

Occurs in excessive production of lactic acid as in cases of severe muscle exercises, shock, or poisoning with cyanide or carbon monoxide.

4) **Administration of ammonium chloride (NH_4Cl):**

NH_4Cl dissociates into ammonium (NH_4^+) and chloride (Cl^-) ions, which leads to acidosis due to:

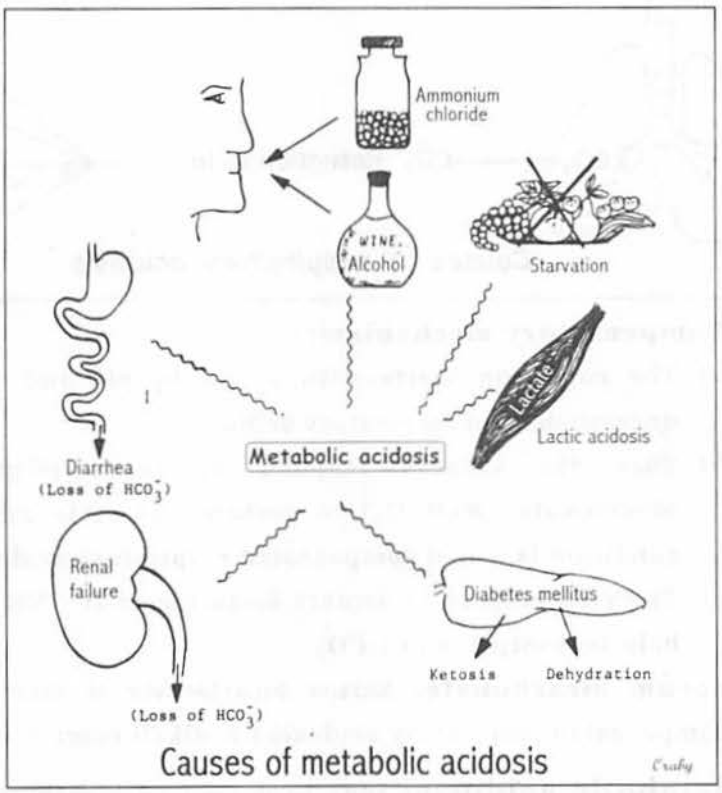
i- Ammonium ion itself is an acid (proton donor).

ii- In the liver, NH_4^+ is converted into urea . Two hydrogen ions ($2H^+$) are liberated for every urea molecule formed.

iii- The chloride ions replace the blood bicarbonate.

5) **High protein diet:** Due to excessive production of phosphoric, sulfuric, and uric acids.

6) **Methanol poisoning:** The liver cell converts methanol to formic acid. This leads to severe acidosis.



b) **Failure to excrete acids: as in chronic renal failure** due to defective excretion of hydrogen ions, associated with defective reabsorption of HCO_3^- ions.

c) **Loss of bicarbonate: as in diarrhea,** where acidosis results from:

1) Loss of bicarbonate from lower small intestine.

2) Dehydration associated with diarrhea.

d) **Renal failure (Renal tubular acidosis):**

1) Where there is reduced Na^+/H^+ exchange in proximal and distal tubules.

2. **Compensatory mechanism:**

a) The condition starts with a fall in pH and it is called: uncompensated metabolic acidosis.

b) This condition is corrected mainly by increased pulmonary ventilation through stimulation of respiratory center by the excess hydrogen ion concentration.

c) Hyperventilation will result in CO_2 wash and decreased carbonic acid and the ratio 20/1 is restored.

3. **Serum bicarbonate:** it is decreased in the metabolic acidosis i.e. alkali reserve is decreased.

III. *Alkalosis*

A. **Definition:** It is a state of a decreased hydrogen ion concentration of the blood i.e. the blood pH is higher than normal (higher than 7.43).

B. **Types of alkalosis:**

1. **Respiratory alkalosis:** Due to a primary decrease in the concentration of carbonic acid content (CO_2 tension) resulting from an increased ventilation.
2. **Metabolic alkalosis:** Due to a primary increase in the concentration of bicarbonate ($NaHCO_3$), resulting from abnormal metabolism.

$$\frac{H_2CO_3}{NaHCO_3} = \frac{1}{20} \longrightarrow \begin{cases} \text{Respiratory alkalosis} = \dfrac{\downarrow H_2CO_3}{NaHCO_3} \\ \text{Metabolic alkalosis} = \dfrac{H_2CO_3}{\uparrow NaHCO_3} \end{cases}$$

C. **Respiratory alkalosis:** due to decreased carbonic acid content of the blood (↓ CO_2 tension).

1. **Causes:**

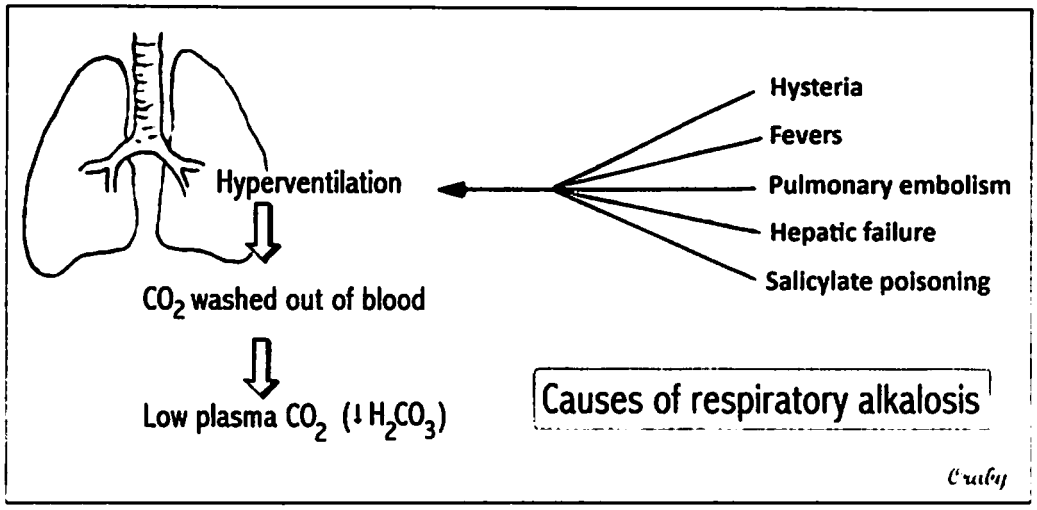
a) Hysterical hyperventilation.

b) Fevers especially lobar pneumonia, meningitis and encephalitis.

c) Pulmonary embolism.

d) Early stage of salicylates poisoning.

e) Hepatic failure.



2. **Compensatory mechanism:**
   a) The condition starts with an increase in pH and it is called: uncompensated respiratory alkalosis.
   b) It is corrected by the kidneys which respond by decreasing renal tubular reabsorption of bicarbonate (HCO_3^-) to restore the ratio 20/1 and it becomes compensated respiratory alkalosis.
   c) There is decreased secretion of H^+ and increased secretion of HCO_3^- and K^+ and the urine becomes alkaline.
3. **Serum bicarbonate:** It is decreased in respiratory alkalosis i.e. alkaline reserve is decreased.

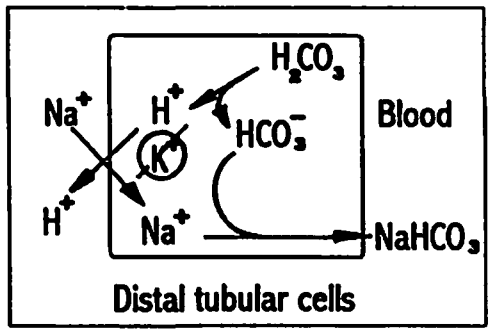
D. <u>Metabolic alkalosis</u>: (Due to increased bicarbonate content of the blood).

1. **Causes:**
   a) **Excessive loss of acids:** as in:
      1) Prolonged vomiting.
      2) Gastric suction (removal of gastric HCl).
      3) High intestinal obstruction (as in pyloric stenosis).
         - In these conditions, loss of chloride ions will lead to chloride deficiency and increase the reabsorption of bicarbonate by the kidneys to restore the electrolyte balance. This is called: hypochloremic alkalosis.

   b) **Administration of excessive amounts of alkalies as in:**
      1) Sodium bicarbonate as in treatment of peptic ulcer.
      2) Potassium citrate used as anticoagulant for blood transfusion.

c) **Potassium depletion (hypokalemia):**

1) **In hypokalemia, K^+ ions moves outside the body cells, driving H^+ ions inside the cells. This results in alkalosis.**



2) **In distal tubule cells of the kidney, either K^+ or H^+ ions are secreted in exchange for Na^+. Therefore in potassium depletion, H^+ -and not K^+ ions- are secreted in exchange for Na^+. Then sodium (Na^+) ions will react with bicarbonate to form sodium bicarbonate, which passess to the blood causing alkalosis.**

d) **Excessive mineralocorticoids activity as in primary aldosteronism and Cushing's syndrome.** The biochemical changes are similar to those occurring in hypokalemia.

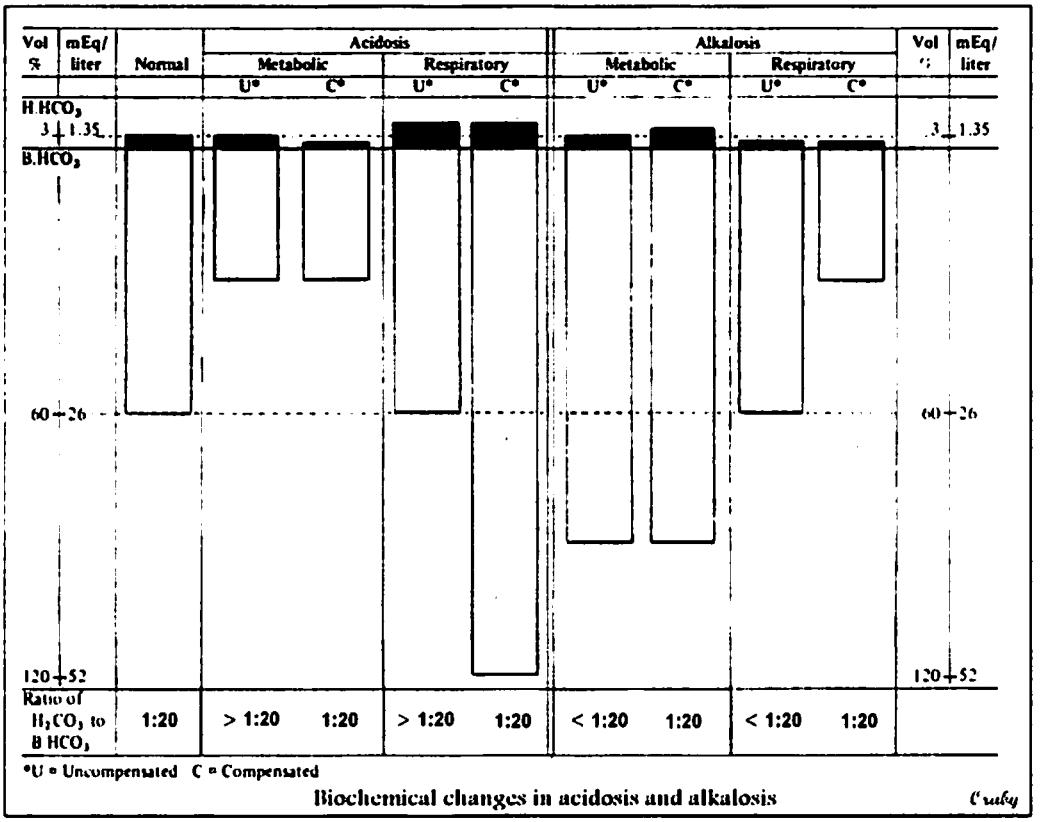
2. **Compensatory mechanism:**
   a) The condition starts with an increase in pH and it is called uncompensated metabolic alkalosis.
   b) This inhibits the respiration, which becomes slow and shallow to increase H_2CO_3 content of the blood, and the condition is called: compensated metabolic alkalosis.
3. **Serum bicarbonate:** It is increased in metabolic alkalosis i.e. alkali reserve is increased.

IV. *Summary of serum bicarbonate in acid base disturbance:*

A. **Normal serum bicarbonate** = 22-30 mmol/ L (average 26 mmol/L).

B. **Decreased serum bicarbonate occurs in:**
   1. Metabolic acidosis.
   2. Compensated respiratory alkalosis.

C. **Increased serum bicarbonate occurs in:**
   1. Metabolic alkalosis.
   2. Compensated respiratory acidosis.



Biochemical changes in acidosis and alkalosis

Role of the kidney in regulation of acid base balance:

I. *Introduction:*

A. The buffer bicarbonate / carbonic acid is normally kept within the ratio of 20 / 1.

B. The carbonic acid (dissolved CO_2) is regulated by respiratory center and lungs.

C. Bicarbonate (HCO_3^-) is regulated by the kidneys. This can be achieved by 2 processes:

1. **The filtered bicarbonate is completely reabsorbed by the tubules:**

   a) **The mechanism** is shown in the following figure.

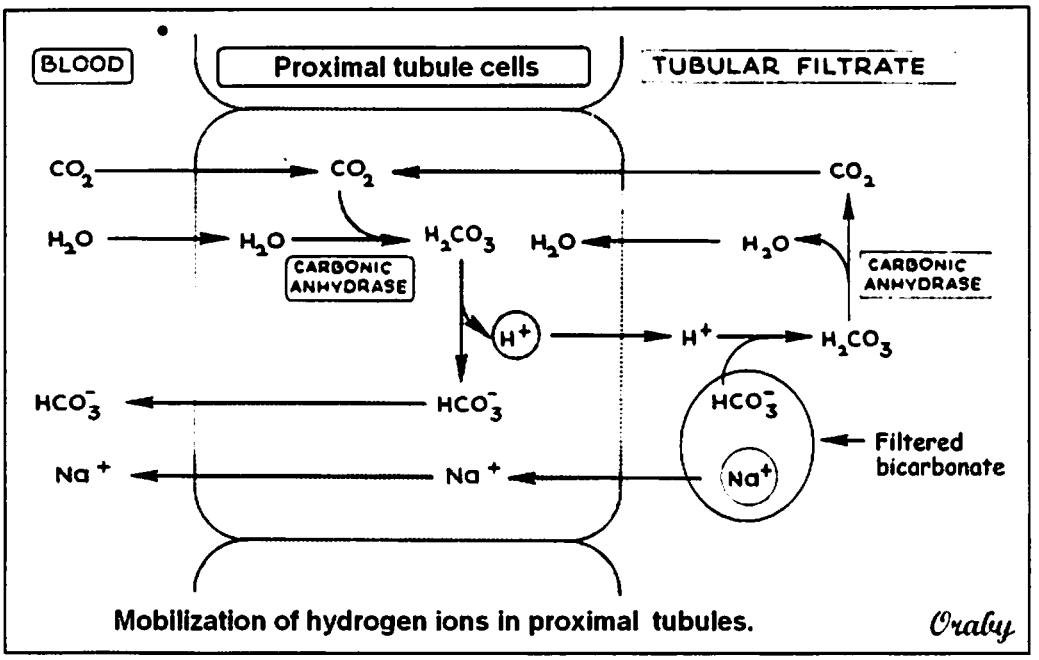
   b) The amount of reabsorbed bicarbonate depends on:

   1) **Concentration of bicarbonate in plasma:**

   - At concentration of plasma bicarbonate below 25 mmol/L the filtered bicarbonate is completely reabsorbed and no bicarbonate appears in urine.
   - Above concentration of 25 mmol/L, excess bicarbonate is excreted in urine.

2) **Concentration of K^+, Cl^- and CO_2 in plasma:**

- Decreased CO_2 tension and increased plasma levels of potassium (K^+) and chloride (Cl^-) inhibit bicarbonate reabsorption and vice versa.

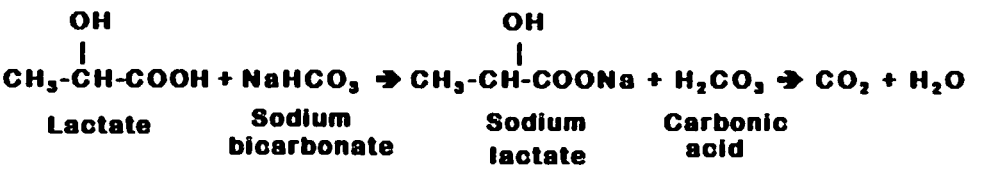


Mobilization of hydrogen ions in proximal tubules.

2. **Regeneration of utilized bicarbonate:**

a) Bicarbonate is regenerated in the distal tubule to replace, that has been utilized by the presence of nonvolatile acids (HCl, H_3PO_4, H_2SO_4 and organic acids) within extracelular fluids as a result of metabolic processes.

b) **Example:**



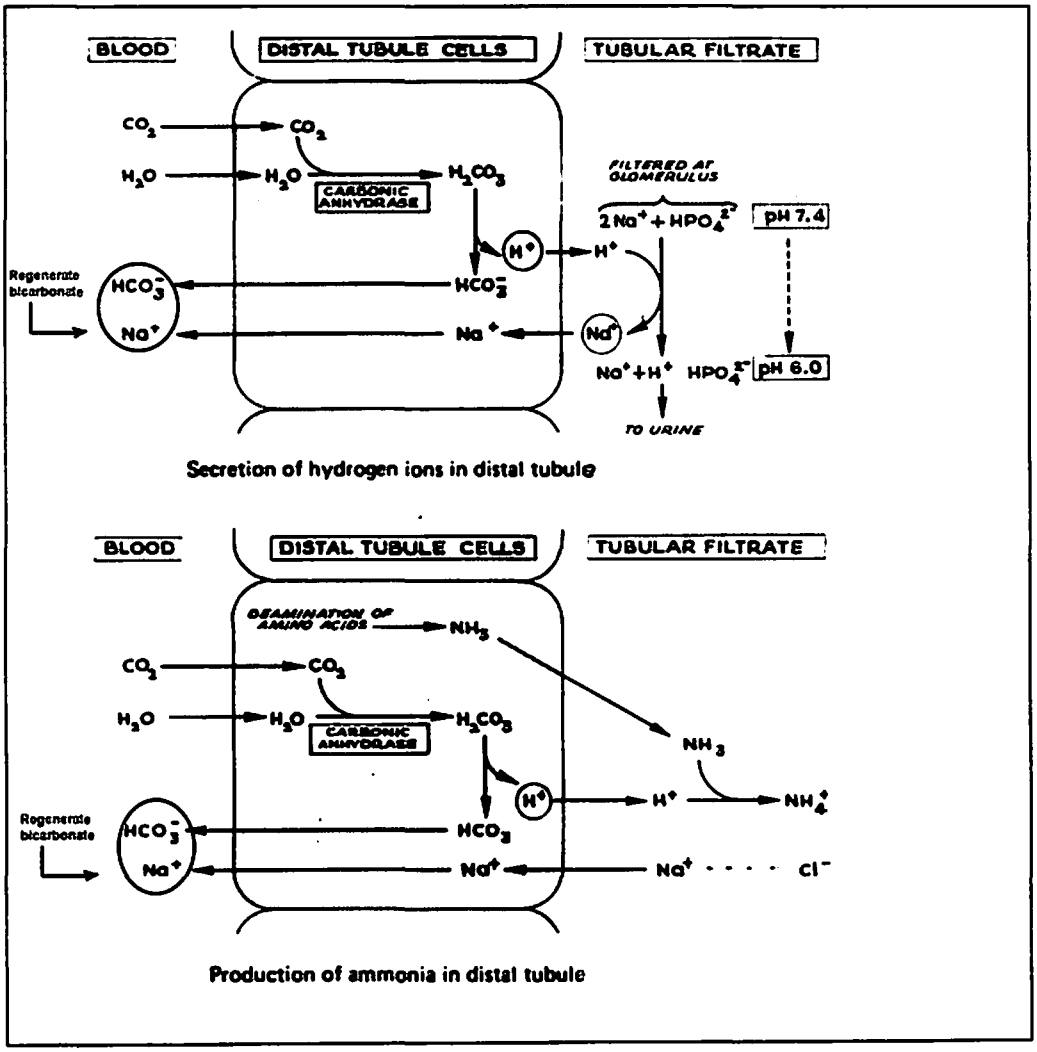
c) **Mechanism of regeneration of bicarbonate:** (shown in the 2 figures below):

The hydrogen ions, which are released from the dissociated carbonic acids inside the tubular cells, are secreted in the lumen of the distal tubules in exchange for sodium ions. These hydrogen ions **are then buffered by 2 mechanisms:**

1) **Conversion of sodium monohydrogen phosphate (Na_2HPO4) to sodium dihydrogen phosphate**

(NaH_2PO_4). This leads to an increase in the acidity of urine (pH 7.4 ➔ 6.0).

2) **Conversion of ammonia** (NH_3), which is derived from deamination of amino acids; mainly glutamine to **ammonium ions** (NH_4^+). Ammonium ions are secreted in the form of ammonium chloride.



Chapter 7 *Metabolism of nucleotides*

I. *Introduction:*

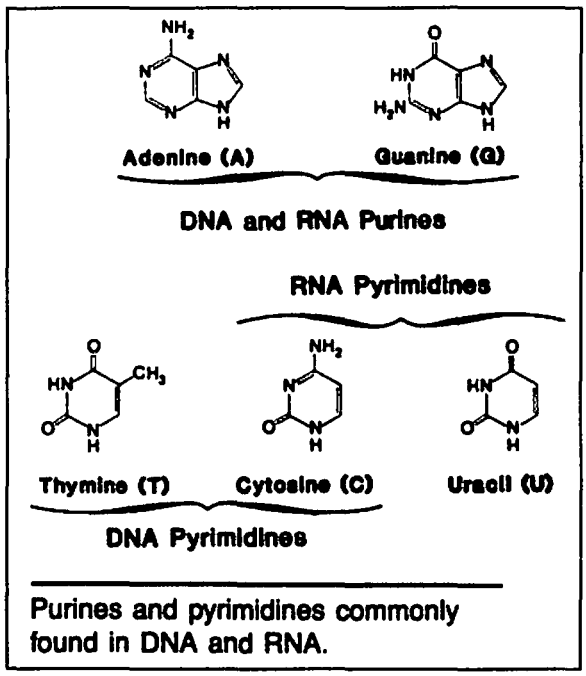
Nucleotides are important intracellular molecules. They are the building blocks of both DNA and RNA. Nucleotides also enter in the structure of many coenzymes. Thus, they are essential for life and health.

II. *Nucleotide structure:*

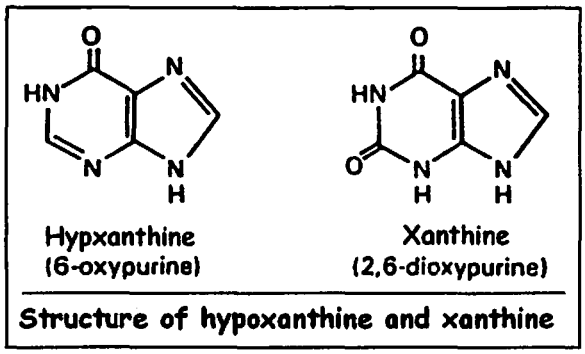
A nucleotide is composed of a nitrogenous base, a pentose sugar and one, two or three phosphate groups.

A. Nitrogenous bases (purines and pyrimidines):

1. Both DNA and RNA contain the same purine bases: **adenine (A) and guanine (G).**
2. Both DNA and RNA contain the pyrimidine **cytosine (C),** but they **differ** in their second pyrimidine base: DNA contains **thymine (T),** whereas RNA contains **uracil (U).** Note that T and U differ only by one methyl group present on T but absent on U).
3. **Bases present in the free state in cells include:**
   a) **Hypoxanthine and xanthine,** which occur as intermediates in the metabolism of adenine and guanine.
   b) **Uric acid,** which is the end product of purine catabolism in human. It is excreted in urine.

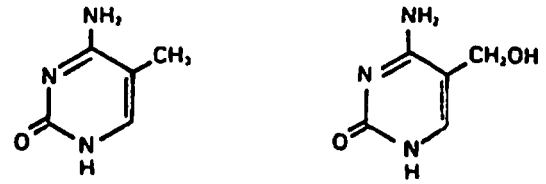


Purines and pyrimidines commonly found in DNA and RNA.

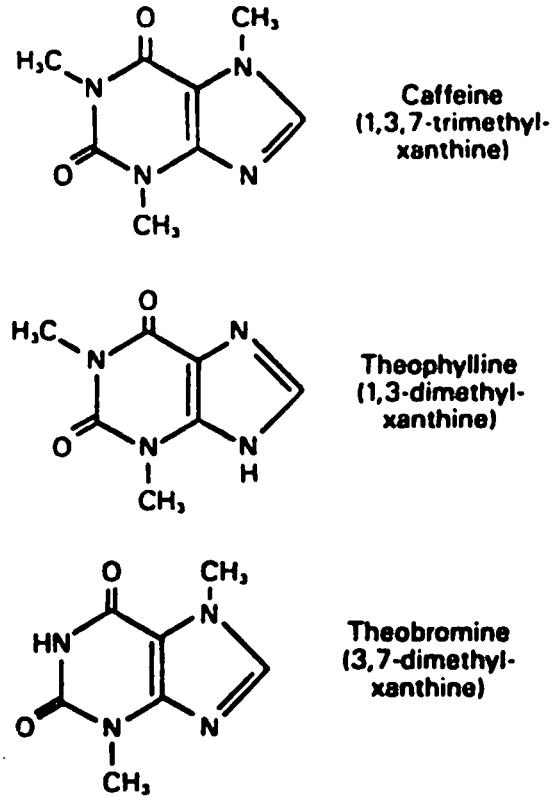


Structure of hypoxanthine and xanthine

4. **Unusual bases:** In addition to the 5 major bases, some uncommon bases may be found in both DNA and RNA e.g. 5-methylcytosine, which may be present in both bacterial and human DNA. Also, 5-hydroxymethylcytosine of viral DNA.
5. **Plant purines:** Plants contain some natural methylated purines. Many of these have pharmacological action e.g.
   a) **Coffee:** Contains caffeine (1, 3, 7 trimethylxanthine).
   b) **Tea:** Contains thiophylline (1, 3 dimethylxanthine).
   c) **Cocoa:** Contains thiobromine (3, 7 dimethylxanthine).



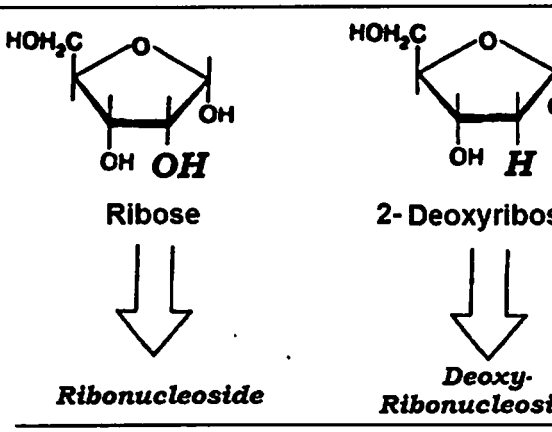
Two uncommon, naturally occuring pyrimidines.



The structures of some methyl xanthines commonly occurring in foodstuffs.

B. Nucleosides:

1. The addition of pentose sugar to base produces a nucleoside.
2. If the sugar is ribose, a **ribonucleoside** is produced. If the sugar is 2-deoxyribose, a **deoxyribonucleoside** is produced.



Pentoses found in nucleotides. Oraby

C. **Nucleotides:**

1. Nucleotides are formed of base, sugar and phosphate.
2. The phosphate group is usually attached by an ester linkage to the 5'-OH of the pentose. Such compound is called 5'-nucleotide.

III. *Biochemical importance (functions) of nucleotides:*

1. They are building blocks of nucleic acids: DNA and RNA.
2. They enter in the structure of ATP, the main source of energy in the cell.
3. They enter in the structure of many coenzymes as NAD^+, $NADP^+$, coenzyme A and FAD.
4. They enter in the structure of cyclic AMP and cyclic GMP, which act as secondary messengers for many hormones.

IV. *Synthetic nucleotide analogs:* Some chemically synthesized analogs of purine and pyrimidine nucleotides are used in treatment of certain diseases. Of these analogs :

A. **5-Fluorouracil, 6-thioguanine and 6-mercaptopurine** are used in the treatment of **cancer.** These compounds inhibit the growth of cancer cells by inhibiting either their enzyme activity or the synthesis of DNA or RNA.

B. **Allopurinol,** a purine analog is used in the treatment of **gout.** It inhibits xanthine oxidase activity, which is essential for uric acid synthesis.

C. **Azathiopurine,** which is catabolized into 6-mercaptopurine, is used during **organ transplantation,** It prevents organ rejection by suppressing immune system.

Metabolism of purine nucleotides

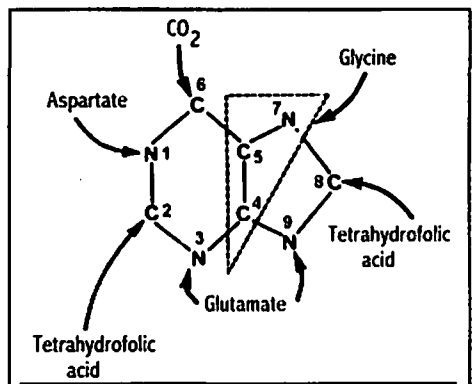
I. *Sources of atoms in purine ring:*

A. **Amino acids:**

1. Aspartate: gives N_1.
2. Glycine: gives C_4, C_5 and N_7.
3. Glutamine: gives N_3 and N_9.

B. **CO_2:** gives C_6.

C. **FH_4 (Tetrahydrofolate):** gives C_2 and C_8.



Sources of the individual atoms in the purine ring.

II. *Synthesis of purine nucleotides:*

- ***De Novo synthesis of purine***
- ***Salvage pathway***
- ***Conversion of ribonucleotides into deoxyribonucleotides.***

They can be synthesized by adding carbon and nitrogen atoms to ribose-5-phosphate (De novo pathway) or can be obtained through salvage pathway by using the preformed purines.

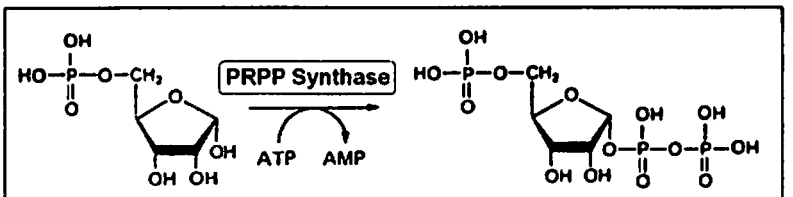
A. De novo pathway:

1. **Steps:** All enzymes involved with purine nucleotide synthesis and degradation are found in the cytosol of the cell. The steps proceed as follows:

a) **Synthesis of 5'-phosphoribosyl-1-pyrophosphate(PRPP):**

1) The transfer of pyrophosphate (PP) from ATP to C-1 of ribose 5-phosphate forms 5-phosphoribosyl-1-pyrophosphate (PRPP).

2) PRPP also participates in the synthesis of pyrimidines and in the salvage reactions of purines.



Synthesis of PRPP.

b) **Synthesis of 5'-phosphoribosylamine:**

1) The synthesis of 5'-phosphoribosylamine from PRPP and glutamine is done by displacement of pyrophosphate from PRPP by the **amide** nitrogen of glutamine.

2) The enzyme PRPP glutamyl amidotransferase is the first enzyme uniquely committed to purine synthesis.

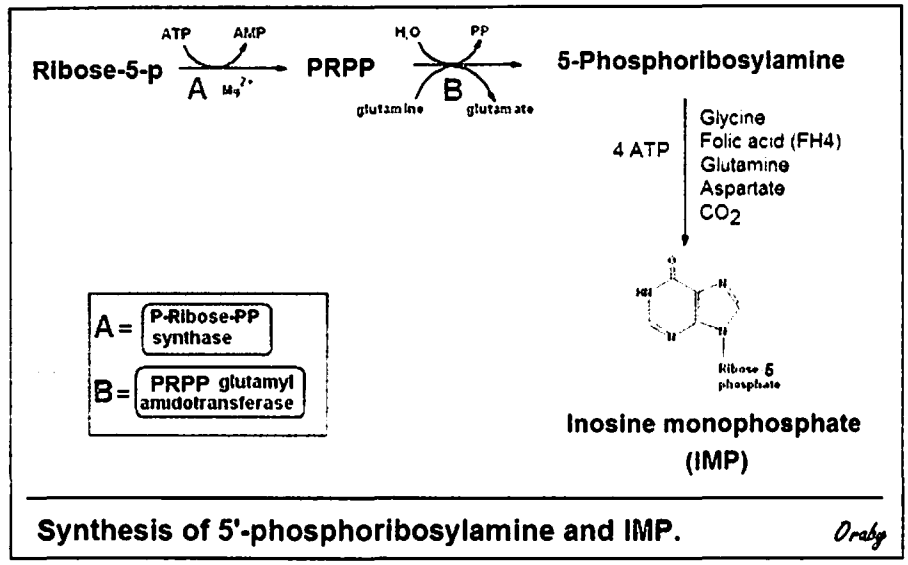
c) **Synthesis of inosine 5'-monophosphate (IMP):**

1) Condensation and reaction of glycine, aspartate, glutamine, Co_2, and folate with 5'-phosphoribosyl-amine form inosine 5'-monophosphate.

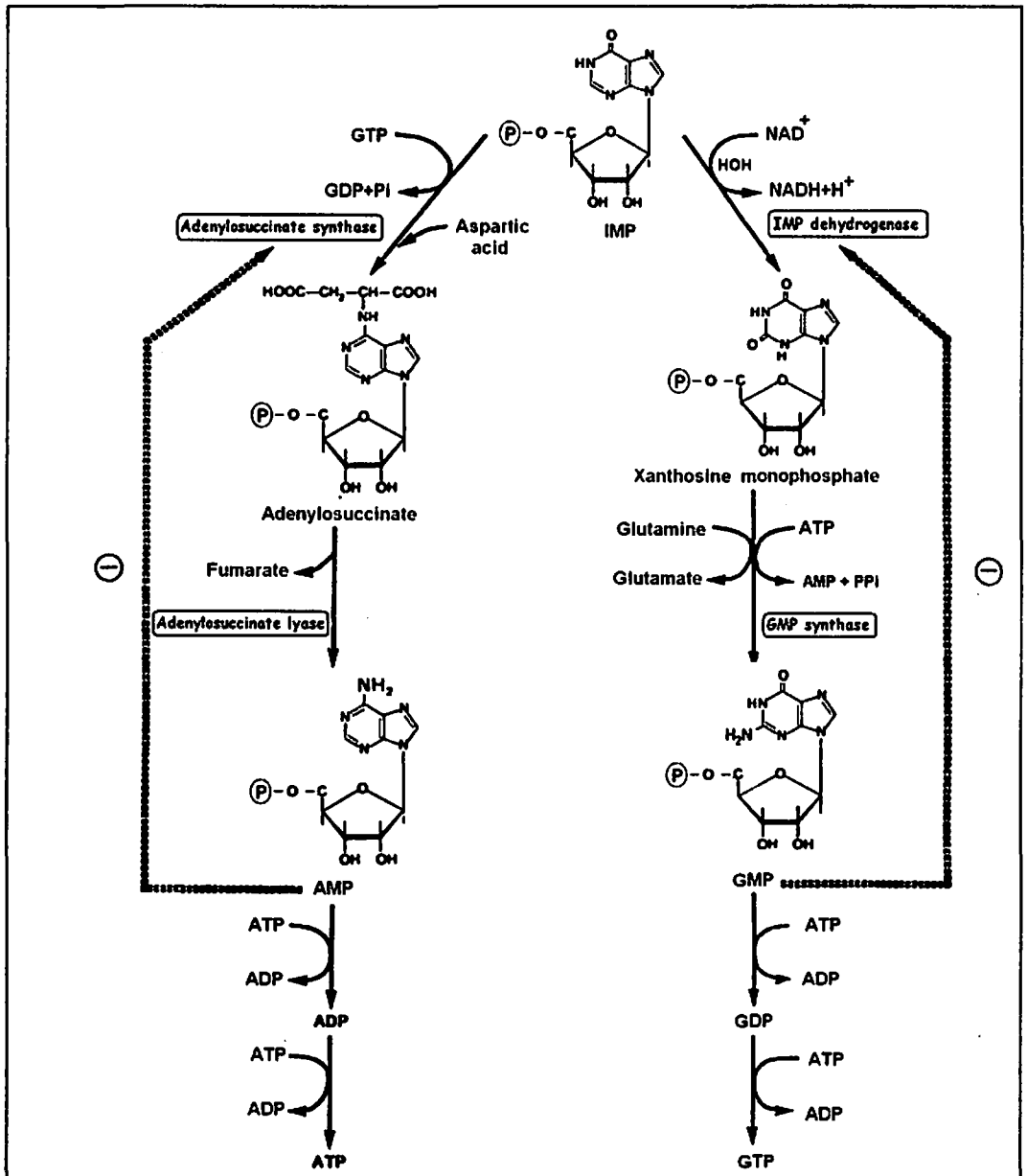
d) **Conversion of IMP to AMP and GMP:**

1) The conversion of IMP to either AMP or GMP utilizes high-energy phosphate bonds.

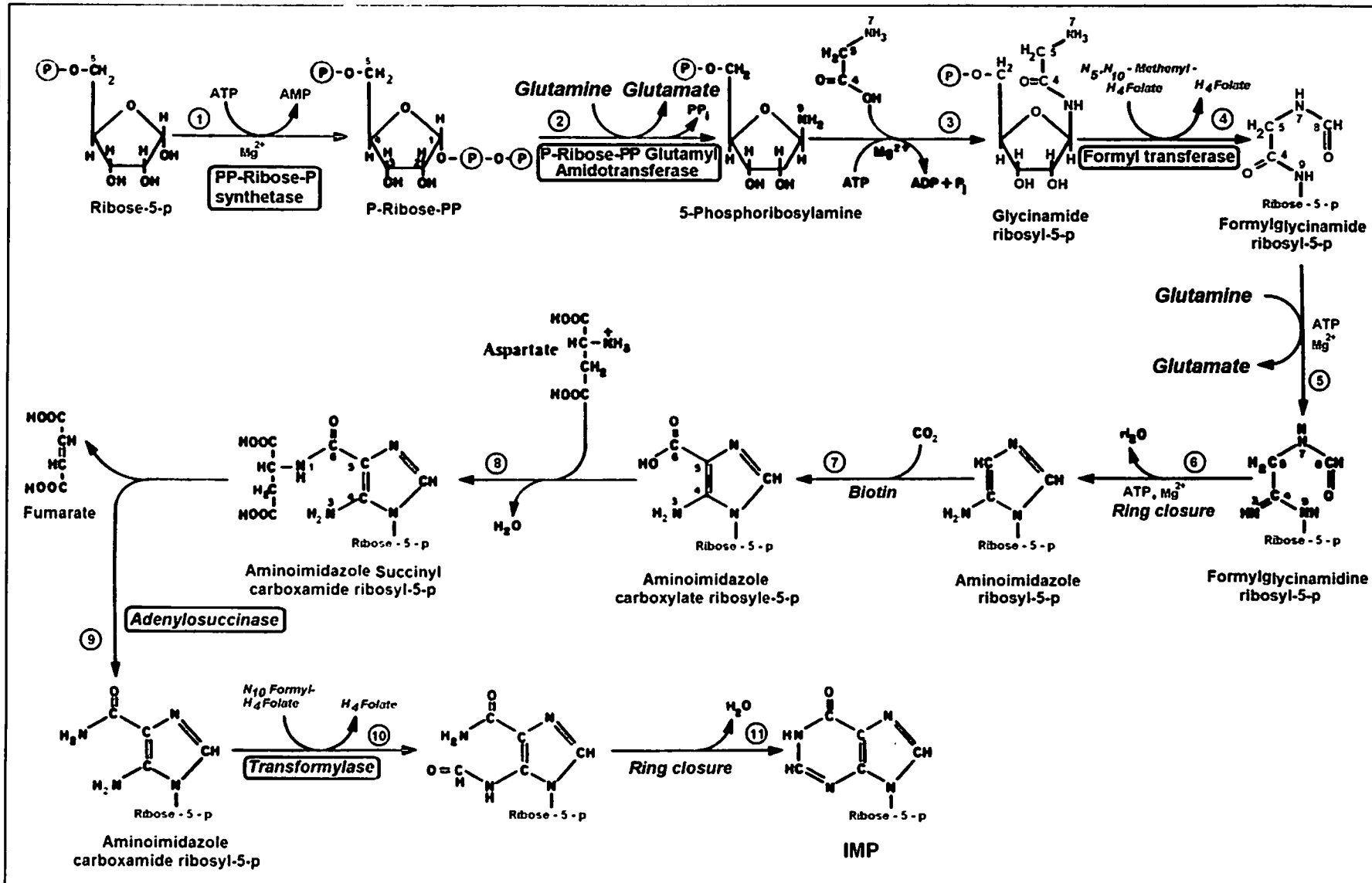
2) The synthesis of AMP requires GTP as an energy source, whereas the synthesis of GMP requires ATP. Also, the first reaction in each pathway is inhibited by the end product of the pathway.



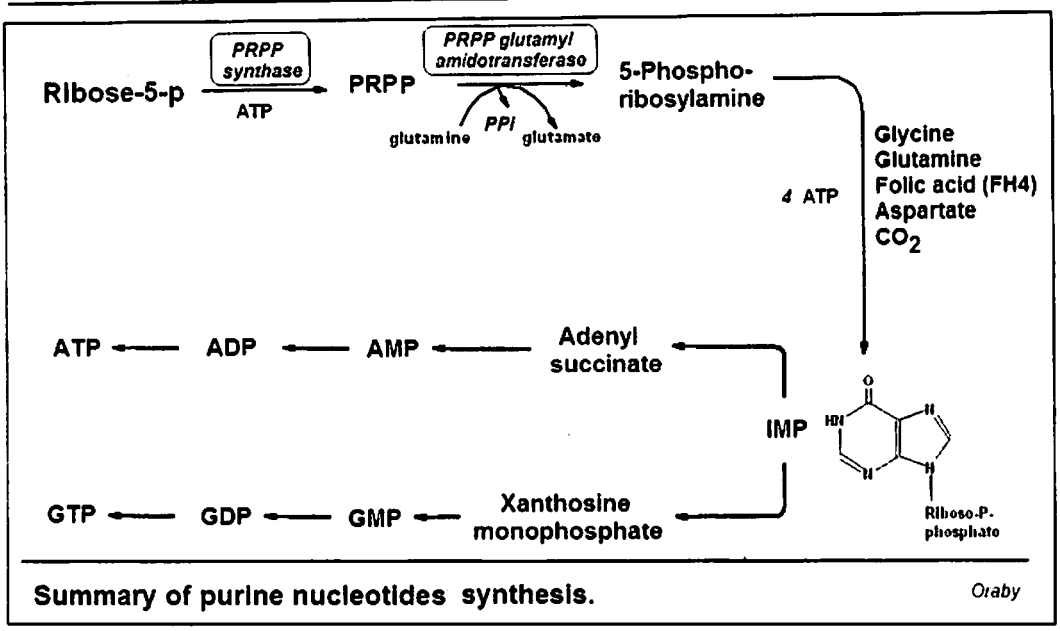
Synthesis of 5'-phosphoribosylamine and IMP. *Oraby*



Conversion of IMP to AMP and GMP showing feedback inhibition.

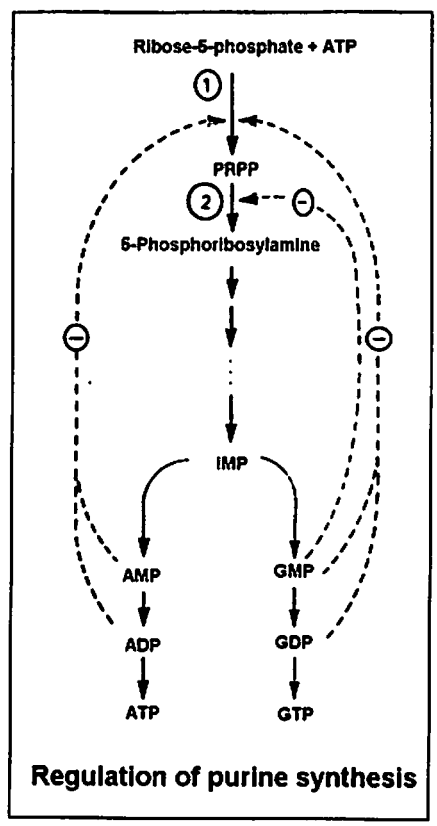


The overall pathway of the de nove purine synthesis.



Summary of purine nucleotides synthesis. *Otaby*

2. **Regulation of purine de novo synthesis:** Purine synthesis utilizes 6 ATP molecules, and requires glycine, glutamine, aspartate and tetrahyrdofolate derivatives. To avoid the loss of unnecessary energy and nutrients, purine synthesis should be regulated to provide the exact needs of purines. **The regulation depends on:**
   a) Availability of ribose-5-phosphate.
   b) Activity of **PRPP synthase** (reaction 1), which is feed back regulated by AMP, ADP, GMP and GDP.
   c) Activity of **PRPP glutamyl amidotransferase** (reaction 2), which is feedback, regulated by GMP and AMP.



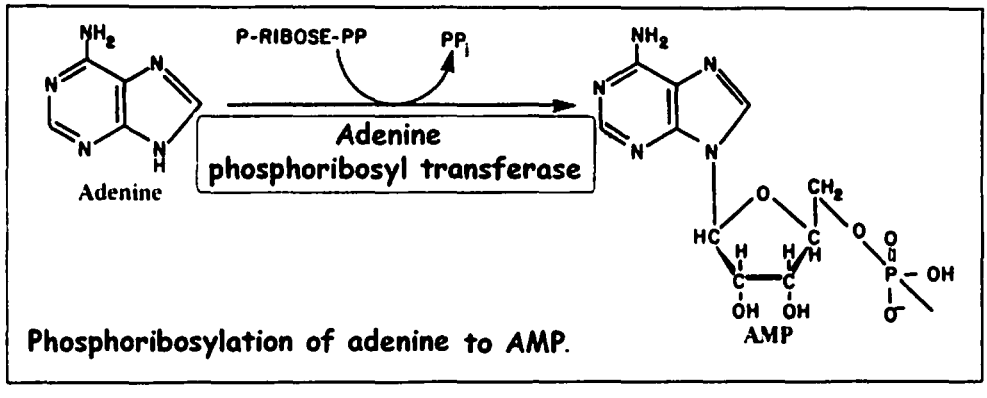
Regulation of purine synthesis

3. **In vitro inhibitors of purine synthesis:**
   a) Inhibitors of purine synthesis are extremely toxic, especially to rapidly growing microorganisms, tumor cells or developing structures such as fetus.
   b) **Examples of purine inhibitors include:**
      1) **Glutamine analogs as azaserine**, which inhibit PRPP glutamyl amidotransferase.

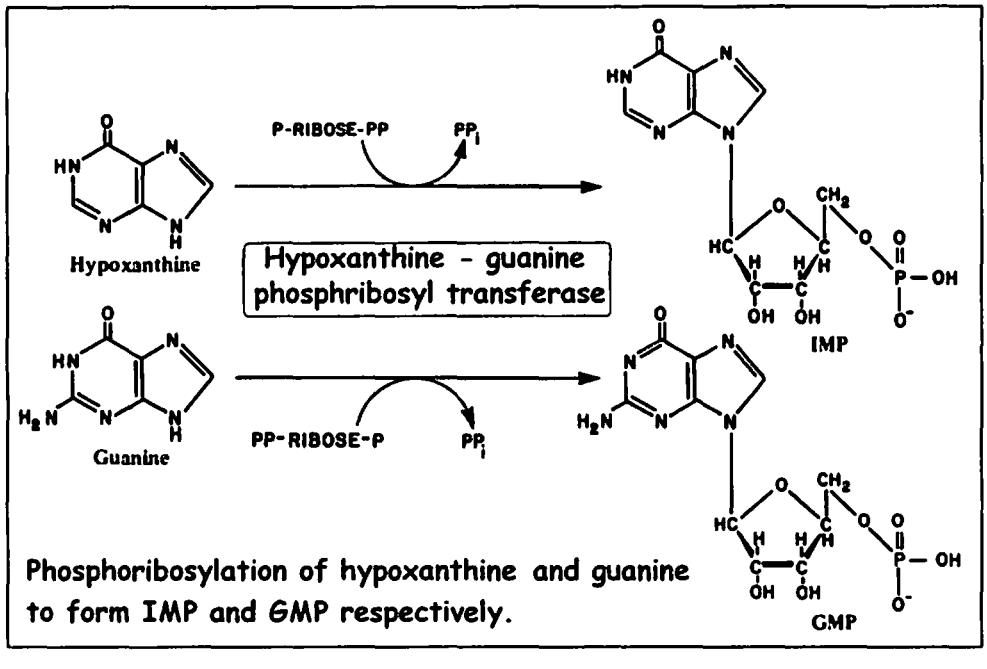
2) **Folic acid analogs as trimethoprim, methotrexate** that competitively inhibit the reduction of dihydrofolate to tetrahydrofolate. Thus these drugs will limit the amount of tetrahydrofolate available for use in purine synthesis.

B. **Salvage pathway for purine nucleotide formation:** The enzymes of de novo synthesis of purines are **deficient** in some tissues like **brain cells, red cells and white blood cells:**

1. These tissues use already synthesized purines for synthesis of purine nucleotides through a pathway called: salvage pathway.
2. **The sources of purines** for this pathway are either bases synthesized in liver or those derived from cellular breakdown.
3. The reactions proceed as shown in the following figures.
4. **Lesch Nyhan syndrom:** It is one type of hyperuricemia (↑ plasma uric acid) resulting form deficiency of **hypoxanthine-guanine phosphoribosyl transferase (HGPRTase)** enzyme (discussed later).



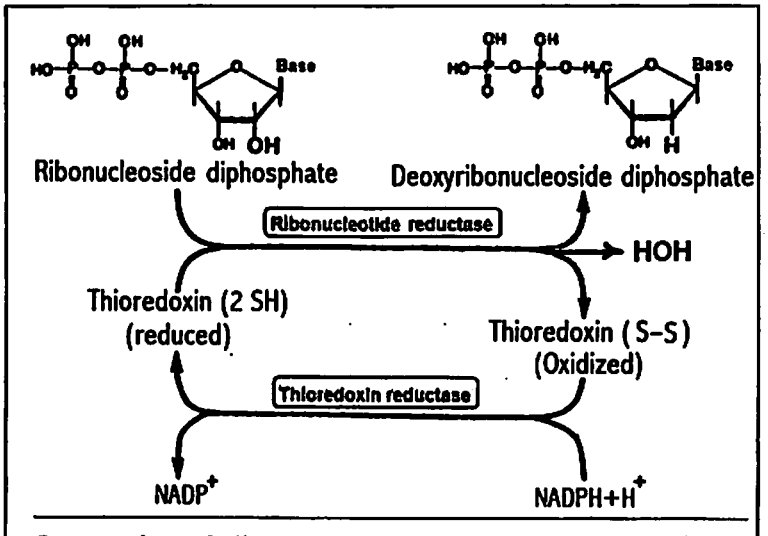
Phosphoribosylation of adenine to AMP.



Phosphoribosylation of hypoxanthine and guanine to form IMP and GMP respectively.

C. Conversion of ribonucleotides to deoxyribonucleotides:

1. The nucleotides synthesized by de novo and salvage pathways are ribonucleotides. These can be used as building blocks in RNA synthesis.
2. The cellular level of **deoxyribonucleotides** is usually very low. They are increased only **at the time of DNA replication.** Deoxyribonucleotides are formed by reduction of the corresponding ribonucleotides, which must be in the diphosphate form.
3. The reaction of conversion needs ribonucleotide reductase and thioredoxin as direct hydrogen donor. Thioredoxin is small protein, which contains 2 cysteine residues. Their two sulfhydryl group (SH) donate their hydrogen atoms forming disulphide bond (S - S).



Conversion of ribonucleotide to deoxyribonucleotide.

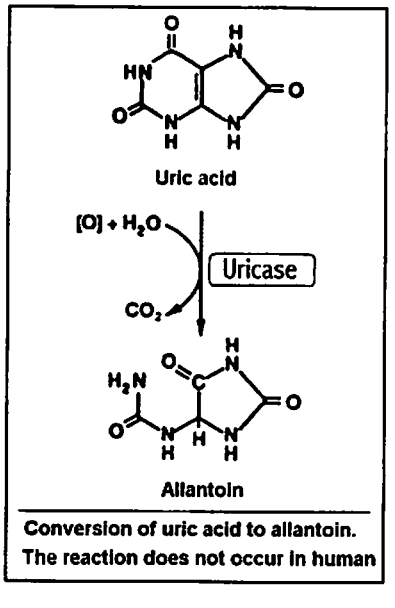
III. *Catabolism of purine nucleotides:*

A. Uric acid is the end product of purine catabolism in man, which is excreted in urine.

B. Net excretion of total uric acid in normal human is about 500 mg / day.

C. Normal human plasma uric acid average **2-7 mg/dl.**

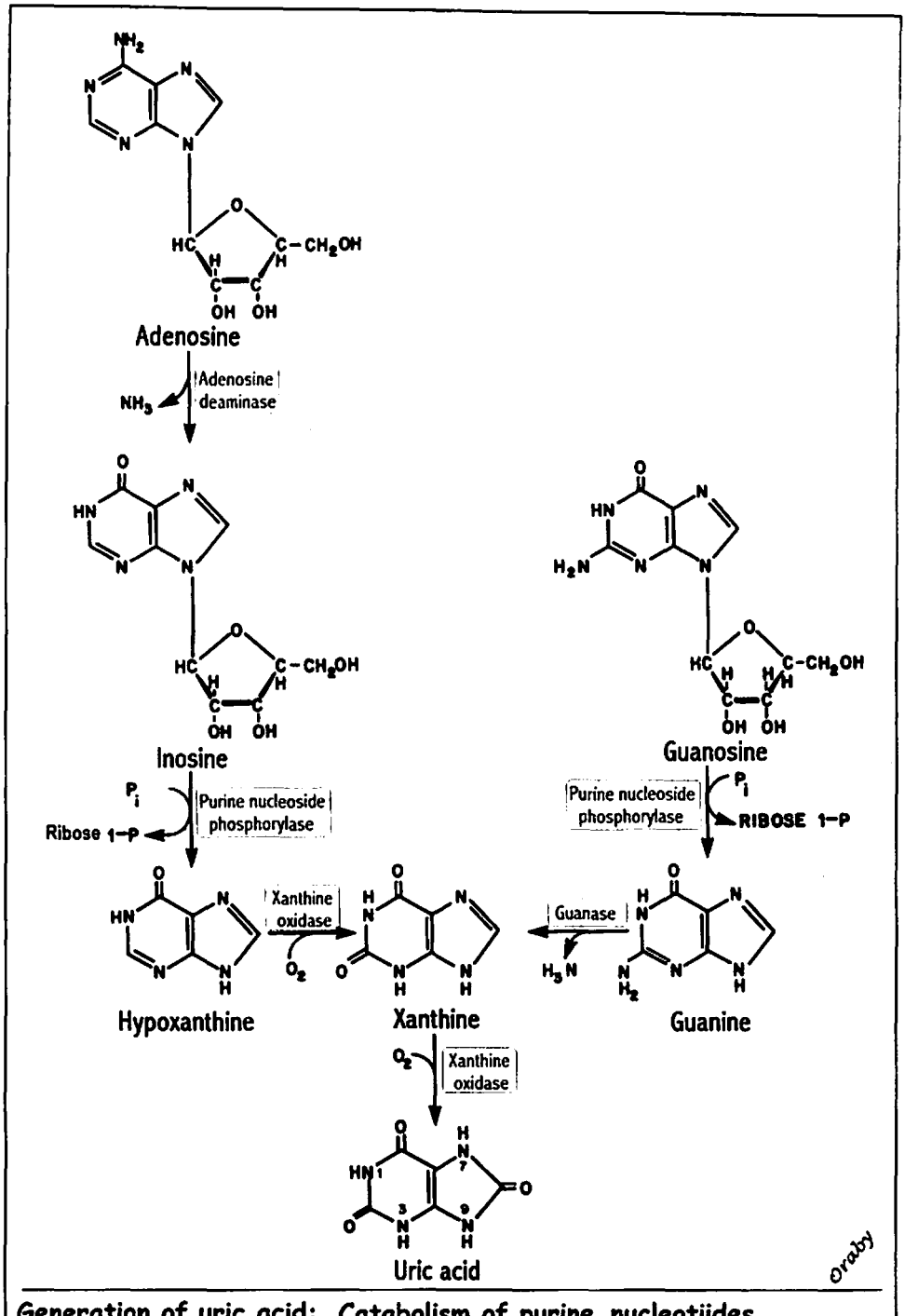
D. In mammals other than higher primates, uric acid is further oxidized into a highly water soluble end product called: **allantion.** This reaction is catalyzed by uricase enzyme, which is lacked in **human.**



Conversion of uric acid to allantoin. The reaction does not occur in human

E. <u>Uric acid crystals and stones</u>: The solubility of uric acid depends upon pH e.g. uric acid becomes insoluble (crystals) at the site of urine acidification (the distal tubules). The more acidity, the more crystals formation → Uric acid stone.

F. Steps in the production of **uric acid** are summarized in the following figure.



Generation of uric acid: Catabolism of purine nucleotiides.

IV. *Disorders of purine catabolism:*

A. Hyperuricemia:

1. **Definition:** Hyperuricemia is a condition in which serum urate level is increased above normal level (2-7 mg/dl) and exceeds its solubility limit.

2. **Causes of hyperuricemia:**

Primary hyperuricemia (enzyme defects):
1- ↑ *PRPP synthase.*
2- ↓ *HGPRTase (Lesch nyhan syndrome).*
3- ↓ *Glucose-6-phosphatase (Von Gierke's disease).*

Secondary hyperuricemia:
1- ↑ The rate of cell division and tissue turnover.
2- ↓ Renal excretion of uric acid.

a) **Primary hyperuricemia:**

1) **Over activity of PRPP synthase**: This leads to purine overproduction and excretion.

2) **↓ (HGPRTase)** hypoxanthine guanine phosphoribosyl transferase enzyme **(Lesch Nyhan syndrome):** This enzymatic defect leads to hyperuricemia as follows:

↓ HGPRTase ➔ Block salvage pathway ➔ ↑ PRPP ➔ ↑ Purines synthesis (de novo synthesis) ➔ ↑ Uric acid formation ➔ Hyperuricemia.

3) **↓ Glucose-6-phosphatase (Von Gierke's disease):**

i- It is one type of glycogen storage diseases due to deficiency of **glucose-6-phosphatase.** This enzymatic defect leads to hyperuricemia as follows:

↓Glucose -6- phosphatase ➔ ↑ Glucose-6-phosphate ➔ Activation of pentose phosphate pathway ➔ ↑ Ribose-5-phosphate ➔ ↑ PRPP ➔ ↑ Purine synthesis and catabolism ➔ Hyperuricemia.

b) **Secondary hyperuricemia:**

1) It is due to **increase the rate of cell division** and tissue turnover as in **cancer and leukaemia.**

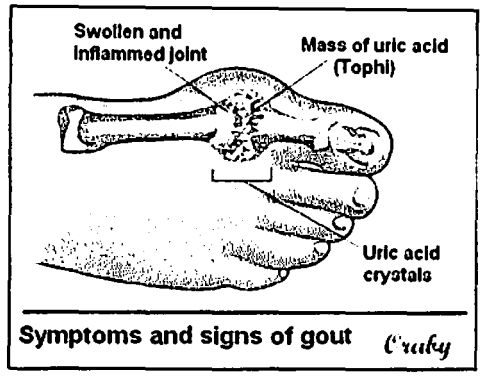
2) **Decreased renal excretion of uric acid:** as in renal failure or due to drugs as diuretics or lead poisoning.

3. **Effect of hyperuricemia → Gout:**

a) ***Tophi formation:*** Increased insoluble urate leads to **crystallization of sodium urate** in soft tissues and joints, which results in formation of deposits called: **tophi.**

1) The tophi cause an **inflammatory reaction** called **gouty arthritis.**

2) **The joints that firstly affected are small joints especially those of big toes.**



Symptoms and signs of gout Oraby

b) **Renal stone:** Deposition of urate crystals in renal tubules may lead to **stone** formation e.g. kidney stone.

c) **(Lesch Nyhan syndrome):** This syndrome is characterized by:

1) Hyperuricemia and gout.

2) Uric acid renal stone.

3) Neurological disorders and mental retardation.

4. **Treatment of hyperuricemia:**

a) Treatment of the cause.

b) **Allopurinol:** It is a structural analogue of hypoxanthine that competitively inhibits xanthine oxidase enzyme → decreasing formation of uric acid.

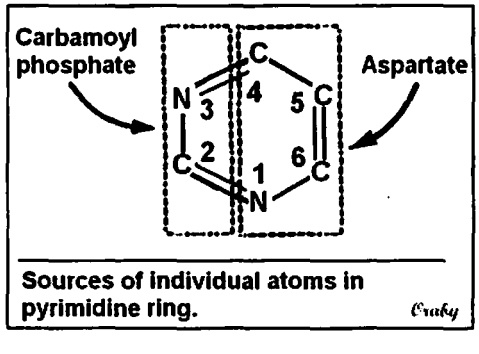
B. Hypouricemia: It is rare condition associated with **xanthine oxidase deficiency.** It is due to either **genetic defect** or **severe liver damage.** It results in excessive excretion of **xanthine and hypoxanthine** and patient may show **xanthine renal stone.**

Metabolism of Pyrimidine Nucleotides

I. *Sources of atoms in pyrimidine ring:*

A. **Aspartate** gives N_1, C_4, C_5 and C_6.

B. **Carbamoyl phosphate** gives C_2 and N_3.

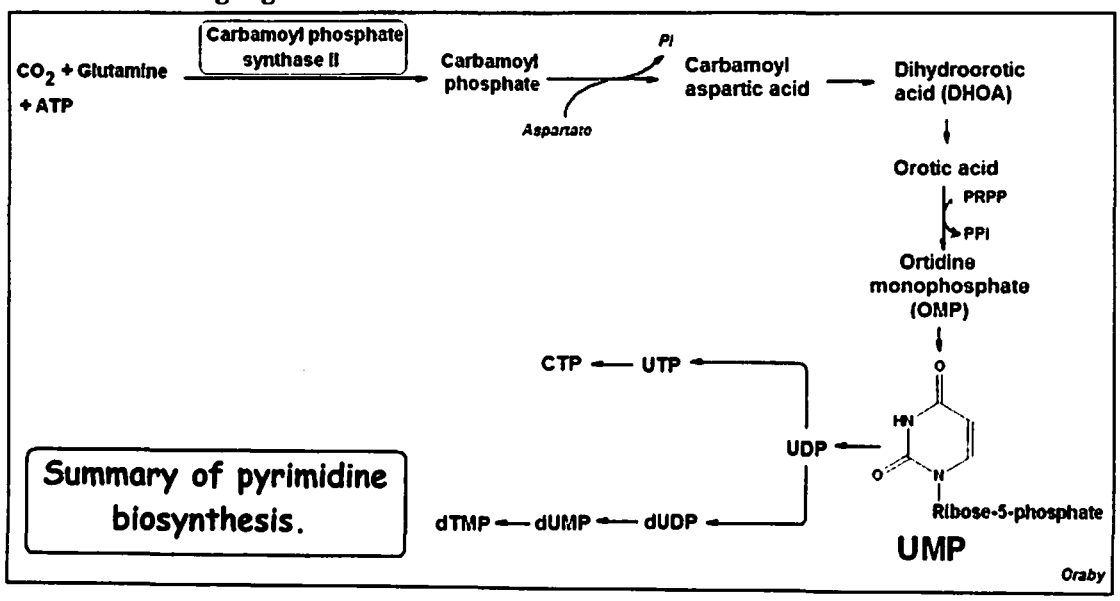


Sources of individual atoms in pyrimidine ring. Oraby

II. *Biosynthesis of pyrimidine nucleotides:*

A. It begins with the formation of carbamoyl phosphate from glutamine, ATP and CO_2. This reaction is catalyzed by cytosolic carbamoyl phosphate synthase II.

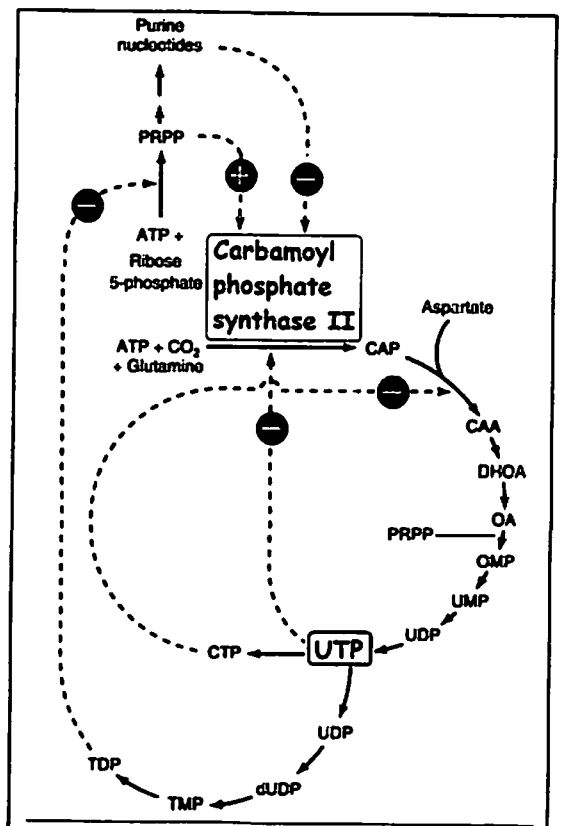
B. **Steps:** The summary of pyrimidine synthesis is shown in the following figure.



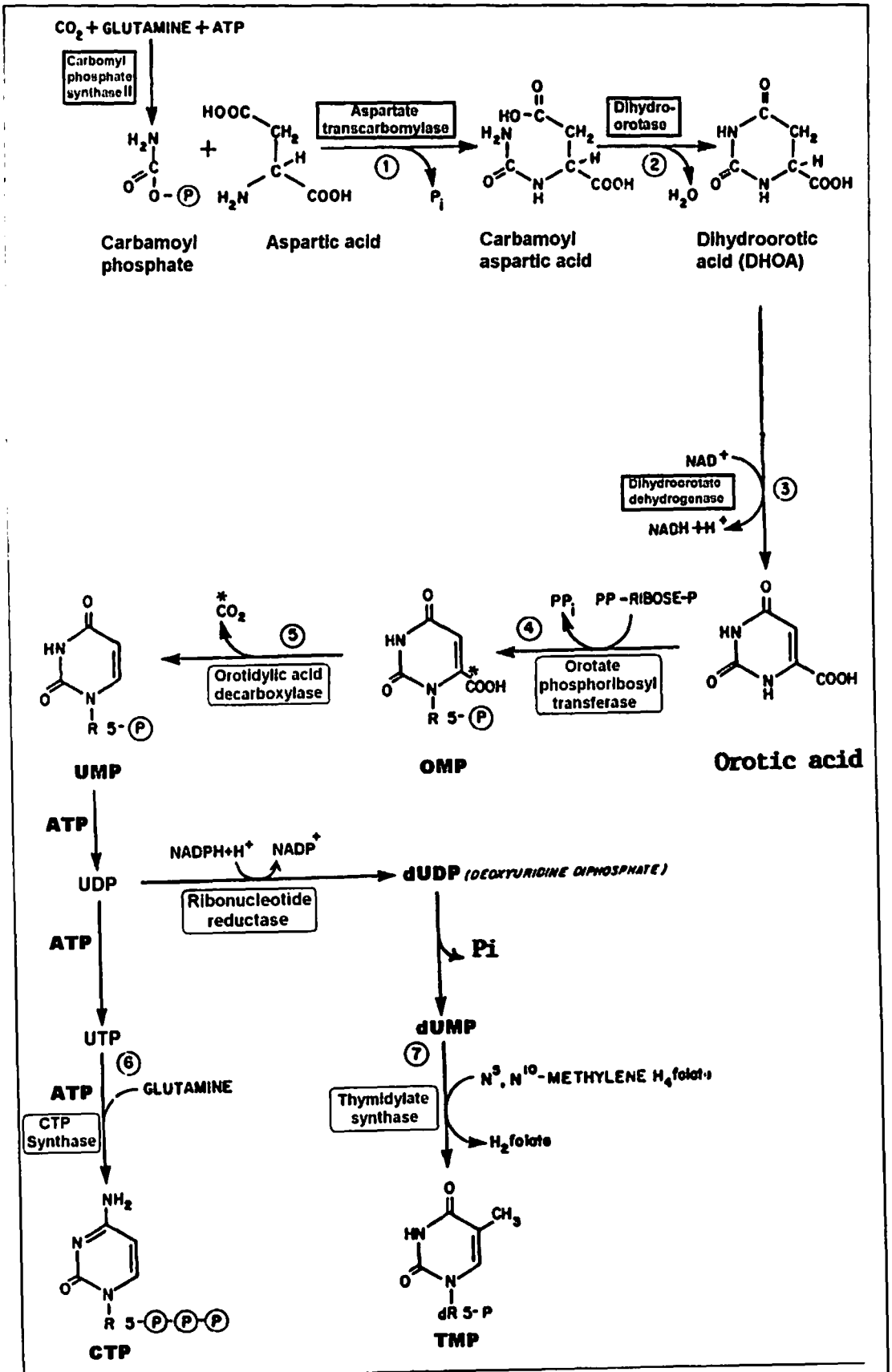
C. **Regulation of pyrimidine synthesis:**

Carbamoyl phosphate synthase II is the key enzyme in this pathway:

1. It is **stimulated** by PP-ribose-phosphate.
2. It is **inhibited** (feedback inhibition) by uracil nucleotides. Also it is inhibited by other purines (see the figure).



Control of pyrimidine nucleotide synthesis.



Biosynthesis of pyrimidine nucleotides.

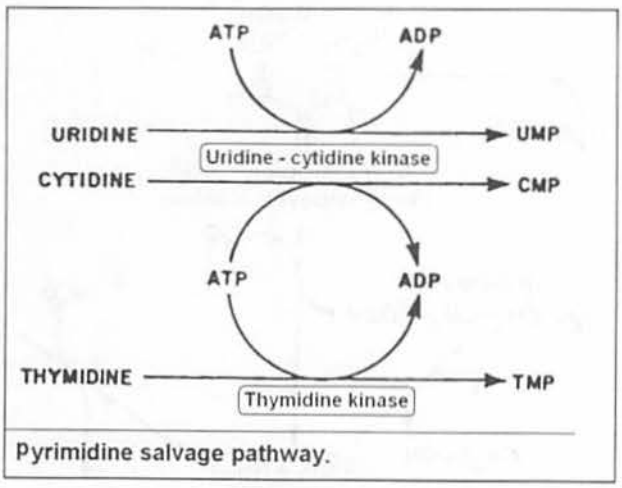
D. Cytosolic carbamoyl phosphate synthase II differs from mitochondrial carbamoyl phosphate synthase I of urea synthesis.

Differences are shown in the following table:

	Mitochondrial carbamoyl phosphate synthase I	Cytosolic carbamoyl phosphate synthase II
Function	Urea synthesis	Pyrimidine synthesis
Site	Mitochondria of liver cells	Cytosol of most tissue cells
Substrates	NH_3, CO_2 and ATP	Glutamine, CO_2 and ATP

E. Pyrimidine salvage pathways:

1. Mammalian cells cannot use **free** pyrimidine bases to form pyrimidine nucleotides.
2. They can convert (salvage) pyrimidine nucleosides; uridine, cytidine and thymidine to their respective mononucleotides.



Pyrimidine salvage pathway.

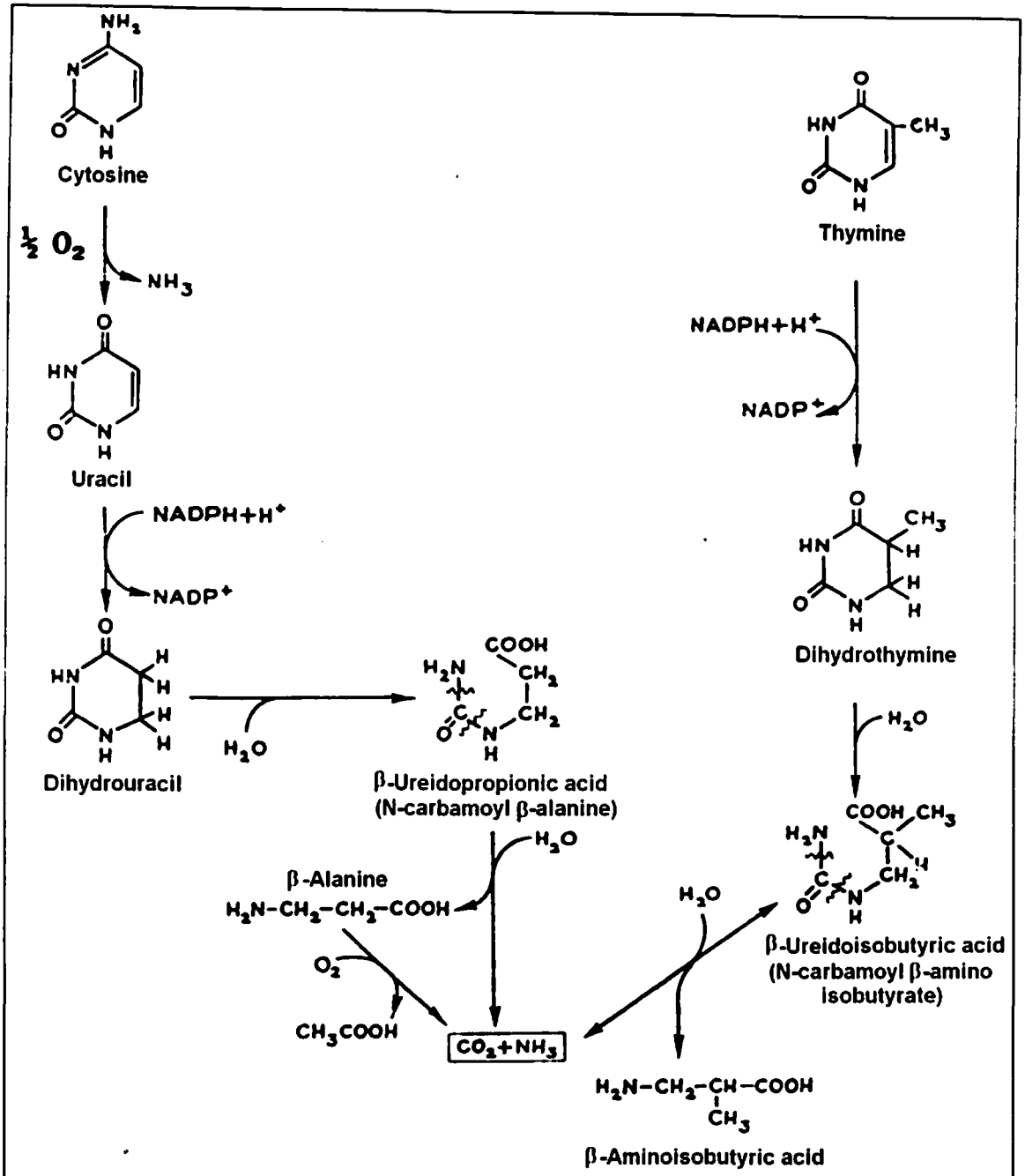
III. *Catabolism of pyrimidine nucleotides:*

A. The catabolism of pyrimidines occurs mainly in the liver.

B. β-Alanine is the major end product of both cytosine and uracil. β-Aminoisobutyric acid is the major end product of thymine.

C. The release of respiratory CO_2 from the pyrimidine nucleus (C_2) occurs in the course of catabolism of all three pyrimidine bases.

D. **Steps:** The overall reactions are summarized as shown in the figure.



Catabolism of pyrimidines.

Chapter 8 *Metabolism of nucleic acids and molecular biology*

I. *Introduction:*

A. *Unlike carbohydrate, protein and lipid, nucleic acids are neither used for energy production nor enter in the structure of the cells. They are used for the* ***storage and expression of genetic information.***

B. *In this chapter, biochemistry of both eukaryotic and prokaryotic cells will be discussed.* ***Eukaryote*** *is an organism whose cells contain limiting membrane around nuclear material e.g. human cells.* ***Prokaryote*** *is an organism whose cells contain no mitochondria and its DNA not enclosed within a membrane and does not undergo mitosis during replication e.g. Bacterial cells.*

II. *Site and functions of nucleic acids:* There are two types of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acids (RNAs):

A. *DNA:*

1. **Site:**
   - *a)* **In eukaryotic cells,** DNA is found in:
     1) The nucleus (nuclear DNA).
     2) In the mitochondria (mitochondrial DNA , mtDNA).
   - *b)* **In prokaryotic cells,**
     1) There is a single chromosome that contains DNA.
     2) There may be also a non-chromosomal DNA in the form of **plasmid** (see later).
2. **Functions:**
   DNA directs the development of the organism through:
   - *a)* Replication (cell division).
   - *b)* Expression of genetic information and protein synthesis (through RNAs).
3. **For mitochondrial DNA:**
   - *a)* **There are 2-10 copies of small circular double stranded DNA.**
   - *b)* **mtDNA replication, transcription and translation of genetic code into proteins occurs inside mitochondria.**

c) mtDNA is responsible for synthesis of enzymes of respiratory chains.

d) Mutation of mitochondrial DNA leads to some diseases e.g. myopathies.

B. RNAs:

1. Site:

a) RNAs that are synthesized in the nucleus perform their functions in the cytosol (ribosomes).

b) RNAs that are synthesized in the mitochondria remain and perform their functions within this organelle.

2. Functions of RNAs:

a) Translation of genetic code (protein synthesis).

b) Some viruses use RNA in either its single or double stranded form as a genetic material i.e. RNA is used instead of DNA.

III. *Structure of DNA:*

A. Building blocks of DNA:

1. DNA is a polynucleotide that contains millions of nucleotides. Each nucleotide is formed of base, sugar and phosphate group. They are covalently linked to each other by **3`,5` phosphodiester bonds.**
2. These bonds join the 5`-hydroxyl group of the pentose of one nucleotide to the 3`-hydroxyl group of the pentose of the next nucleotide through phosphate group (figure 8.1).

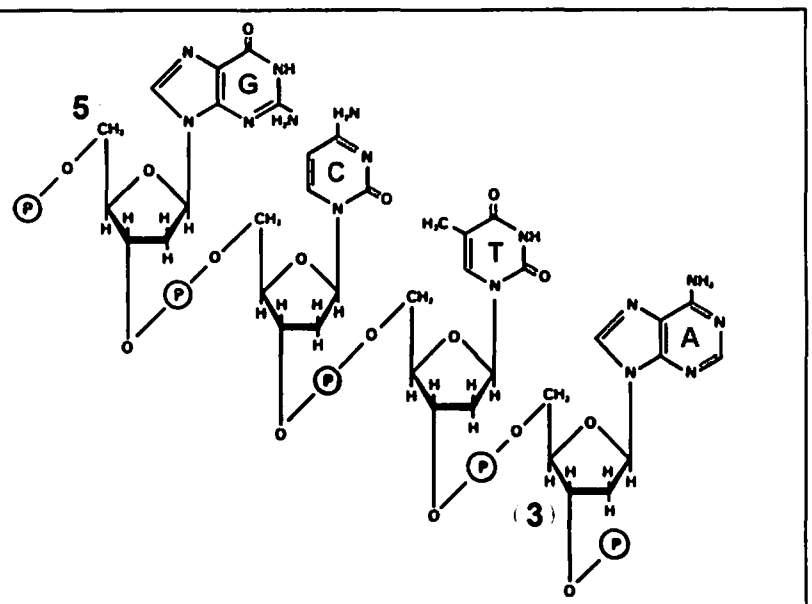


Figure 8.1: A segment of a structure of DNA molecule in which the purine and pyrimidine bases : [adenine (A), thymine (T), cytosine (C) and guanine (G) are held together by phosphodiester bonds.

3. The resulting DNA chain has a polarity i.e. the 2 ends of the polymer are different, with a 5` end that is not attached to another nucleotide and 3` end that is also unattached (figure 8.3).
4. The bases located along DNA strand are always read from the 5` end of the chain to the 3` end. For example, the sequence of nucleotides shown in figure 8.1 is read "guanine, cytosine, thymine and adenine".

B. Structure of DNA:

1. DNA exists as a double stranded molecule in which the two strands form a **double helix.** There is some exception of few viruses that contain single stranded DNA.
2. The 2 strands are complementary not identical.
3. The 2 strands of DNA molecule are **antiparallel** i.e. one strand runs in the 5` to 3` direction and the other strand runs in the 3` to 5` direction (figure 8.2).

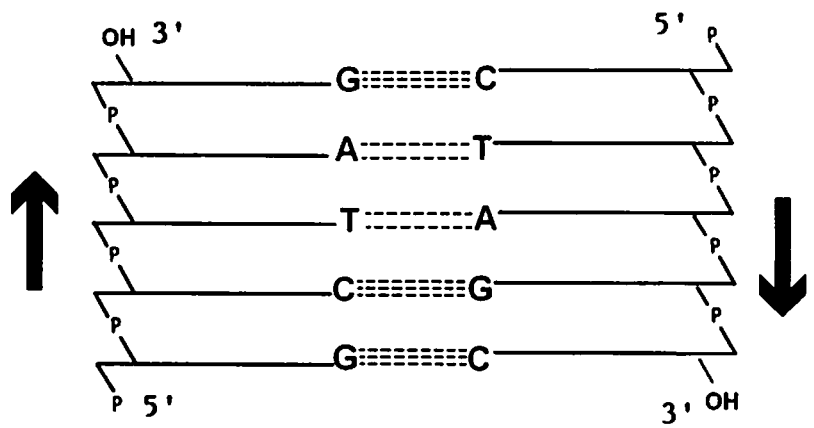


Figure 8.2: Double stranded DNA with antiparallel strands.

NOTE: In some organisms such as bacteria and many DNA viruses, the 2 ends of the DNA molecule are joined to create a closed circle with no terminus. This eliminates all free 3` and 5` hydroxyl and phosphoryl groups (figure 8.12).

4. The 2 strands of DNA molecule are coiled around a **common axis** (figure 8.3).
5. **Structural forms of DNA:**
   a) Three structural forms of DNA have been described:
      1) **The B-form:** Described by Watson and Crick in 1953. It is a **right-handed helix.**
      2) **The A-form:** It is also **right handed helix**, but more compact than B-form.

3) **The Z-form:** It is **left handed helix.**

b) The most common type of DNA helix is the **classic B form** (figure 8.3), where:

1) The hydrophilic ribose phosphate molecules form the **backbone** outside the DNA molecule.
2) The hydrophobic bases are arranged inside, **perpendicular** to the axis of the helix.
3) The overall structure resembles a twisted ladder.
4) The spatial relationship between the two strands in the helix creates a **major** (wide) **groove** and a **minor** (narrow) **groove.**

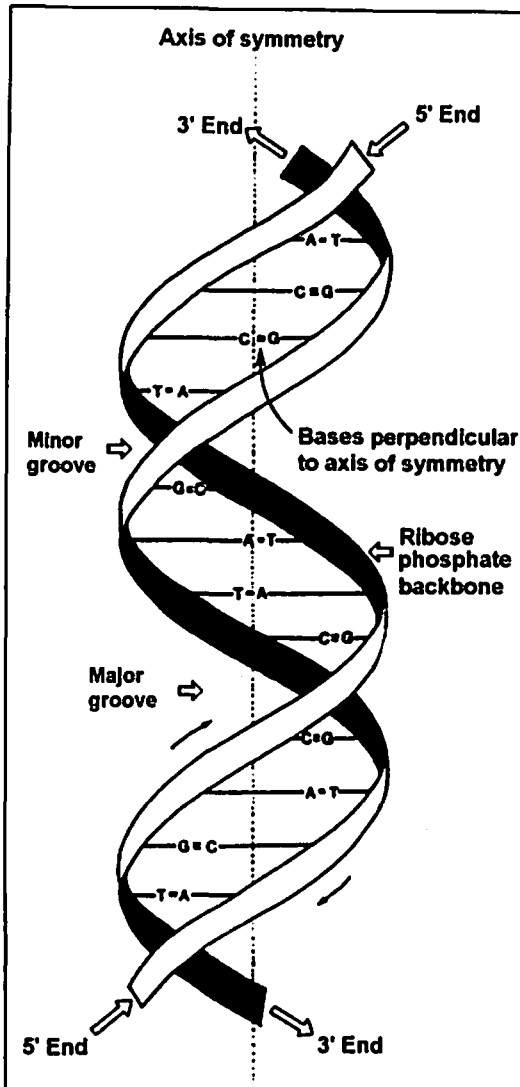


Figure 8.3 : DNA double-helix illustrating some of its major structural features.

6. Pairing rule:

a) The bases of one strand of DNA are paired with the bases of the second strand so that an adenine(s) **(A)** are always paired with thymine(s) **(T),** while a cytosine(s) **(C)** are always paired with guanine(s) **(G).**

b) Therefore one strand of DNA double helix is always **complementary** and not identical to the other strand (figure 8.4).

c) The base pairs are held together by hydrogen bonds: **two** between A and T, and **three** between G and C (figure 8.5). Thus G≡C bonding is stronger than A=T one.

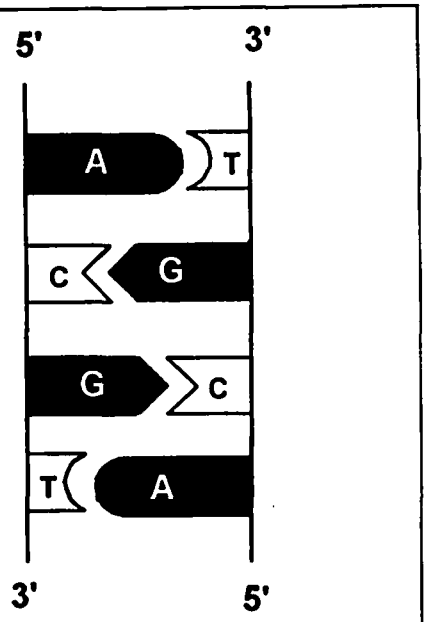


Figure 8.4: Two complementary DNA sequences.

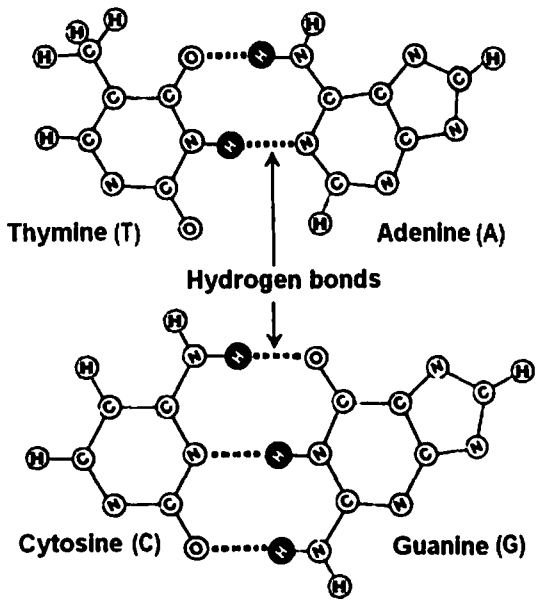


Figure 8.5 : Hydrogen bonds between complementary bases.

C. DNA denaturation, annealing and hybridization:

1. Denaturation:

a) **Definition:** Denaturation is the separation of the two strands of DNA i.e. disruption of hydrogen bonds between the paired bases without affecting the phosphodiester bonds.

b) **Causes of denaturation:**

1. Change of pH **e.g. alkalies.**
2. **Heat:** about 100° C.

c) **Melting temperature (Tm):**

1. It is the temperature at which half of the helical structure is lost.
2. Because there are only 2 bonds between A and T but 3 between G and C, DNA that contains high concentration of A and T denaturates at a lower temperature than G and C rich DNA.

2. Renaturation (annealing):

a) It is the reformation of base pairs and complementary strands of DNA come back together.

b) Renaturation occurs if double stranded DNA are heated and then the temperature is slowly decreased under appropriate conditions ➔ Renaturation.

3. Hybridization:

If a single strand of DNA bases pair with complementary bases of another strand of DNA or RNA → New hybrid DNA molecule.

Remember:
- **DNA denautration** is the separation of the 2 strands of DNA by disruption of hydrogen bonds.
- **Causes:** 1) High temperature (100°C) 2) Alkalies

IV. *DNA organization:*

A. Chromosomes:

1. These are **nucleoproteins**, formed mainly of DNA and basic proteins.
2. In man they are 46 in number.
3. They bear genes that act as functional units of heredity.
4. They contain about 3 billions (3000,000,000) base pairs.
5. They are capable of reproducing its physical and chemical structure through successive cell divisions (replication).

B. Telomeres:

1. **Functions:** telomeres protect the end of the chromosome from deterioration.
2. **Location:** telomeres are located at the ends of the chromosomes.
3. **Structure:** telomeres are composed of DNA and proteins. They consist of short, repeat TG-rich sequences. Human telomeres have variable number of repeats of sequences 5′-TTAGGG-3′, which can extend for several kilobases.

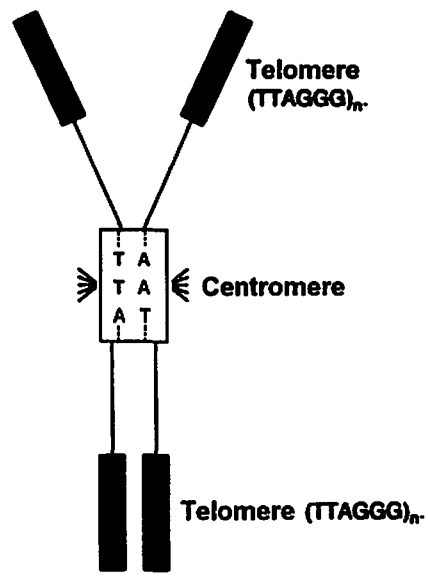
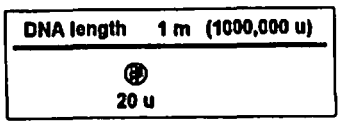


Fig. 8.6

Each chromosome end terminates in a telomere, which consists of a long series of repeat sequences. In humans this sequence is $(TTAGGG)_n$.

4. **Telomerase** is the enzyme responsible for telomeres synthesis and thus for the length of the telomeres.
   a) **Telomerases** are responsible for stability on the ends of chromosomes. They are essential for proliferating normal cells.
   b) **By age,** telomeres become **shorter** and telomerase become **less active.** This may explain in part the process of **aging.**
   c) **Telomerases** are also highly active in cancer cells and in cells with high proliferation rate.

C. Chromatin:



1. It is the **chromosomal material** that is formed of a condensed DNA-protein complex (the DNA found in a single human cell has a total length of 1 meter, and a typical human cell is only 20 micrometers (μ) long. For DNA to be packed into such a small space, it must be condensed into a compact structure. A number of proteins carry out this condensation and the condensed DNA-protein complex is called **(chromatin).**
2. **Chromatin is consisted of:**
   a) Very long **double stranded DNA** molecule.
   b) **Histones:** which are basic proteins.
   c) **Protamines:** which are also basic proteins.
   d) Small quantity of **RNA.**

D. Histones:

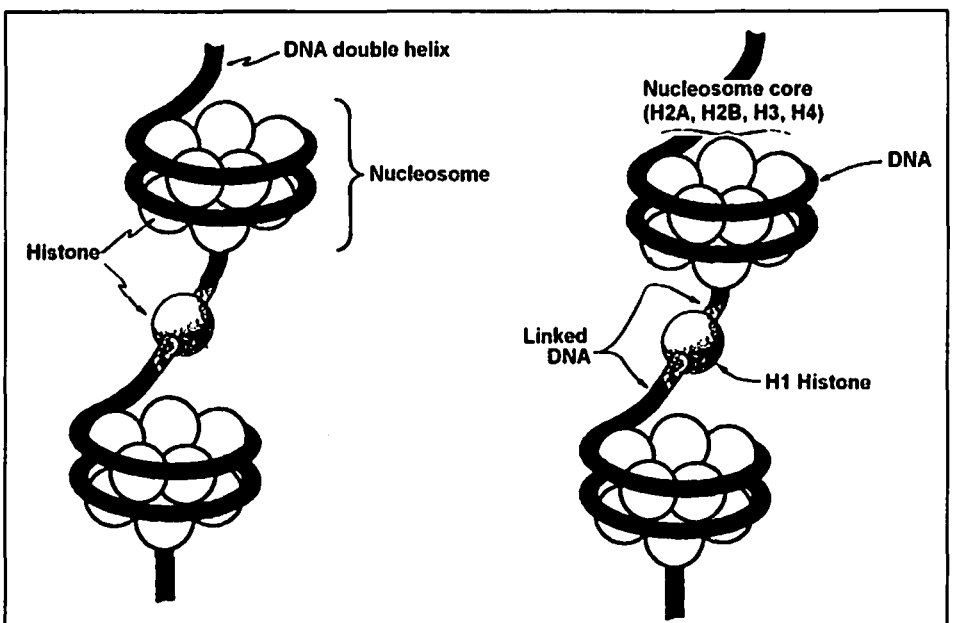


Figure 8.7: Organization of human DNA.

1. Histones are small proteins and are positively charged at physiological pH due to their high content of histidine, lysine and arginine. Because of their positive charges, the histones form ionic bonds with the negatively charged DNA.
2. **Nucleosomes:** Histones are arranged in structure units called nucleosomes; which resemble beads on string formed of DNA (figure 8.7).
3. **There are 5 major classes of histones: H1, H2A, H2B, H3 and H4.** They fall into two main groups:

a) **First group:** of histones forms the **nucleosome** core, which is surrounded by a segment of DNA molecule. This core formed of 8 molecules (2 molecules of H2A, 2 molecules of H2B, 2 molecules of H3 and 2 molecules of H4).

b) **Second group:** includes H1 only. It appears to bind to the DNA chain between the nucleosome beads.

E. Genome:

1. Is the total number of genes within one mature cell of an organism.
2. The human genome contains about **20,000 to 25,000** genes that constitute the human genome. These genes are present throughout the human genome along the **23 pairs** of chromosomes.
3. The genome of a **gamete** (sperm or ovum) is **haploid** i.e. the number of chromosomes is the half the number in somatic (diploid) cell. The number in human gamete is 23 chromosomes.
4. The genome of somatic cell is **diploid** i.e. it contains twice the normal gametic number of chromosomes. In man, the somatic cell contains **23 pairs** of same (homologous) chromosomes. In each pair, one chromosome is derived from father **(parental)** and the other is derived from mother **(maternal).**

F. Gene:

1. Genes are pieces of DNA, which occupy a specific positions **(locus)** on it.
2. Genes are the fundamental units of hereditary.
3. Each gene is a part of DNA sequences that contains genetic information coding for synthesis of one polypeptide (protein).
4. **Types of genes:**

a) **House keeping genes:**

1) These are genes, which are essential for life of the cell.
2) They are integral parts of the cells e.g. hexokinase gene that is essential for glucose metabolism.

b) **Differential (specific function) genes:**

1) These are genes, which are essential for performance of specific functions and so differ in each organ according to the function e.g. genes specific for liver functions will only be expressed in the liver cells.

G. Alleles:

1. These are forms of a gene occupying the same locus on a homologous chromosomes.
2. Each character (e.g. specific type of protein) is controlled by a pair or a series of genes that occupy the same position (locus) on homologous chromosomes. Each one of these genes is known as **allele** i.e. gametic variant.
3. A pair of alleles may be identical, and is called **homozygous** or different, and is called **heterozygous.**
4. The two alleles give the cell its **genotype** e.g. the alleles code for blood group A may have one of two genotypes: AA (homozygous) or AO (heterozygous).
5. One of the two alleles determines any character, which is called: **dominant allele.** The other one is called: **recessive allele.** The dominant allele (character) gives the cell its **phenotype** e.g. the phenotype of both blood group genotype AA and AO is **A.**

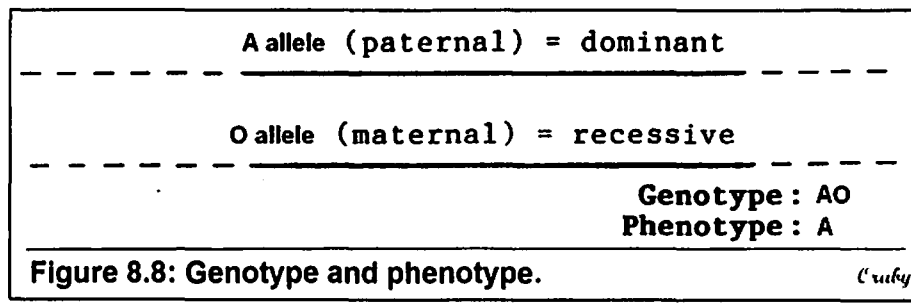


Figure 8.8: Genotype and phenotype.

V. *Structure of ribonucleic acids (RNAs):*

A. Types of RNA: There are three major types of RNA: messenger RNA **(mRNA)**, ribosomal RNA **(rRNA)** and transfer RNA **(tRNA).** Like DNA, all three types of RNA are long, unbranched molecules composed of mononucleotides joined together by phosphodiester bonds.

B. RNAs differ from DNA in:

1. They are **smaller** than DNA.
2. They contain **ribose** instead of deoxyribose.
3. They contain **uracil** instead of thymine.

C. The three types of RNA also differ from each other in: size, function and special structural modifications:

1. **Messenger RNA (mRNA):** It is one strand RNA, composed of 400 – 4000 nucleotides:

a) **Function:** mRNA carries the genetic information from the DNA and is used as a template for protein synthesis.

b) Eukaryotic mRNA (figure 8.9) has **special structural characters;** which are:

1) Long sequence of adenine on the 3` -end of RNA chain **(poly A-tail).**

2) **Cap on the 5`-end.** This cap is consisted of 7-methylguanosine attached through triphosphate linkage.

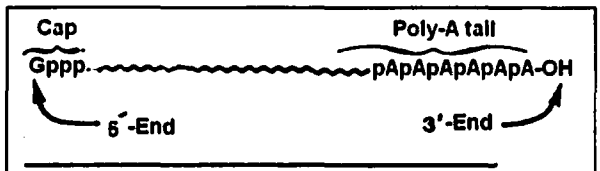


Figure 8.9: Structure of messenger RNA.

2. **Transfer RNA (tRNA):** It is the smallest of RNA molecules. tRNAs have between 73 and 93 nucleotides.

a) There is at least one specific type of tRNA molecule for each of 20 amino acids commonly found in proteins.

b) tRNA molecules have extensive interchain base pairing as shown in figure 8.10.

c) Each tRNA serves as adaptor molecule that carries its specific amino acid to the site of protein synthesis (the mechanism will be mentioned later in protein synthesis).

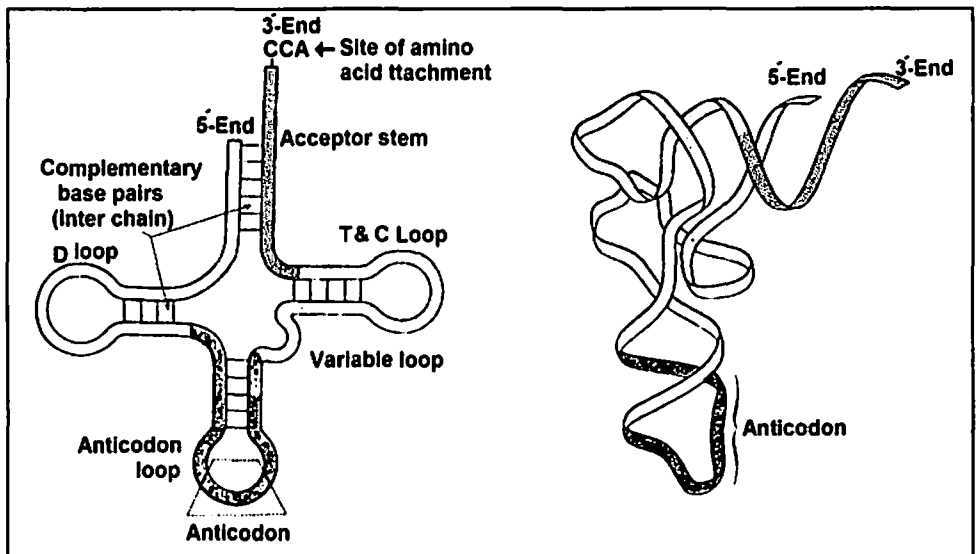


Figure 8.10: structure of transfer RNA.

3. **Ribosomal RNA (rRNA):**

a) **In prokaryotes and eukaryotic mitochondria:** There are three species of rRNA: 23S, 16S and 5S.

b) **In eukaryotic cytosol:** There are four species of rRNA: 5S, 5.8S, 18S and 28S.

> ***Note:*** *S is the abbreviation of sedimentation velocity in* ***Svedberg units.*** *These units depend on sedimentation coefficient i.e. they depend on both size and molecular weight of particles.*

c) **Function of rRNA:** They combine with a number of different proteins forming the ribosomes. Ribosomes serve as the sites of protein synthesis.

D. Nucleases:

1. These are enzymes acting on DNA and RNA and chemically cleaving the phosphodiester bonds.
2. Types:
   - *a)* **Endonucleases**: are nucleases that can hydrolyze internal internucleotide linkages.
   - *b)* **Exonucleases**: are nucleases that act only on terminal nucleotides linkages. These are further classified into :
     1) **5'-Exonucleases** are nucleases that act on the 5' end of nucleic acid chain.
     2) **3'-Exonucleases** which act on the 3' end of nucleic acid chain.

Summary:

- **The unit of the nucleic acids** is a nucleotide. Each nucleotide is formed of: a nitrogenous base + pentose sugar + Phosphate.
- DNA contains the bases adenine (A), guanine (G) cytosine (C) and **thymine (T),** while RNA contains the bases adenine (A), guanine (G) cytosine (C) and **uracil (U).**
- **Deoxyribose** is present in DNA, and **ribose** is present in RNA.
- **Nucleic acids** are polynuceotides. The nucleotides are joined together by 3', 5' phosphodiester bonds.
- **The genetic information** is present in the sequence of polynuceotide chains.
- In DNA, 2 polynucleotide chains **are joined (through hydrogen bonds) by** pairing between their bases (adenine with thymine and guanine with cytosine), and they form a double helix. One chain runs in a 5' to 3' direction and the other runs 3' to 5'.
- DNA molecules **interact with histone** to form strands of nucleosomes, which wind into more tightly coiled structure.

DNA Replication (DNA Synthesis)

I. *Introduction:*

A. Before a cell divides to form 2 daughter cells, it must first make two copies of its genetic material so that the parent copy can be distributed to each daughter cell.

B. The process by which DNA is copied is called **semi conservative**. This means that after replication, each of daughter DNA molecules will contain:

1. **One old strand:** i.e. one parent strand is conserved.

2. **One new strand:** This is synthesized from free nucleotides present in the nucleus.

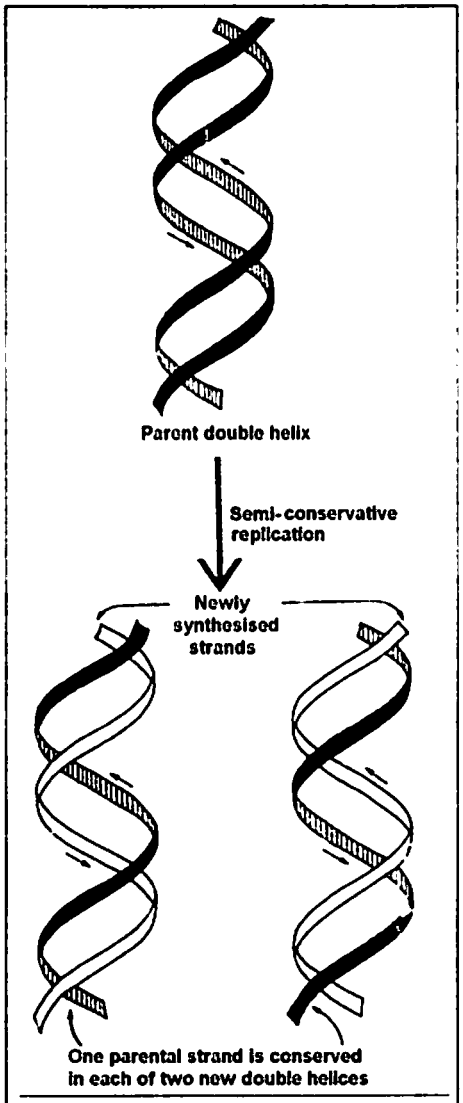


Figure 8.11 Semiconservative replication of DNA.

C. **Enzymes for DNA replication:** These are **DNA polymerases:**

1. **In prokaryotes** e.g. bacteria, there are three species of DNA polymerase; I, II, III.
   a) **DNA polymerase I** catalyzes DNA replication and repair.
   b) **Polymerase II** catalyzes proofreading and repair.
   c) **DNA polymerase III** catalyzes mostly replication of DNA.
2. **In eukaryotes** e.g. human cell, there are 5 species of DNA polymerase: **α, β, γ, δ and ε** (their function will be explained later).

D. **Origin of replication:**

1. **In prokaryotes** e.g. E.coli, DNA replication begins at a single special site called: **the origin of replication** or **oriC.** OriC consists of unique sequence of bases that is recognized by an enzyme system responsible for initiation of replication.
2. **In eukaryotes,** replication begins at a **multiple sites** along the DNA helix. This provides a rapid replicating mechanism for the very long eukaryotic DNA molecule (figure 8.12).

E. **Replication fork:** as the two strands unwind and separate, they form the "V" shape where active synthesis occurs (figure 8.12). This region called the replication fork:

1. The replication fork moves along the DNA molecule as synthesis occurs.
2. Replication of double stranded DNA is **bidirectional** i.e. moves in both directions from 5′ to 3′ direction (figures 8.12 and 8.15).

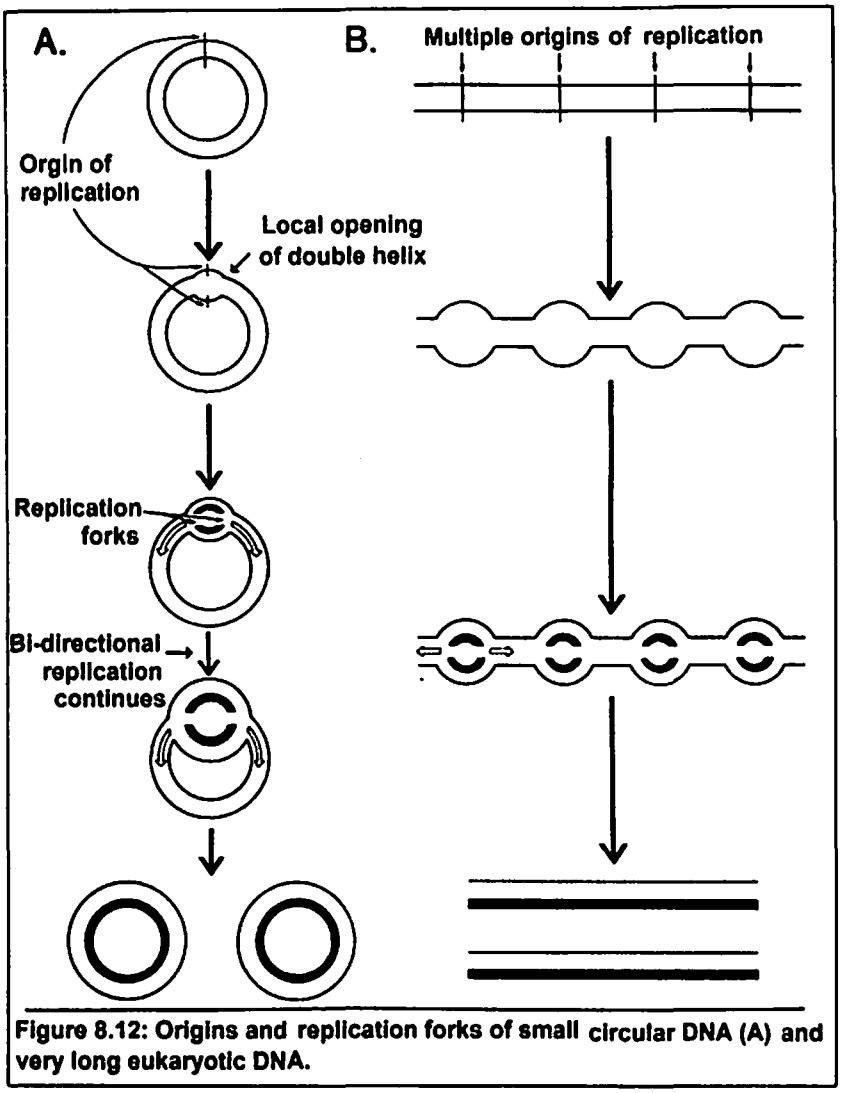


Figure 8.12: Origins and replication forks of small circular DNA (A) and very long eukaryotic DNA.

II. Strands separation (unwinding of parental DNA):

During replication, the double stranded DNA molecule **(duplex)** is separated into 2 strands and each is used as a template for the synthesis of a new complementary strand.

. Proteins responsible for separating the 2 DNA strands:

These proteins are:

1-Helix-destabilizing (HD) proteins.
2-Helix unwinding proteins.
3-Topoisomerases

1. **Helix-destabilizing (HD) proteins:** They bind non-enzymatically to a single stranded DNA, without interfering with the abilities of the nucleotides to serve as templates. **Their functions are:**
   a) They keep the two strands of DNA separated in the area of replication, thus providing the necessary single-stranded template.
   b) They protect DNA from nuclease enzymes that cleave single stranded DNA.

2. **Helix unwinding proteins,** also called DNA helicase or rep protein:
   a) Its function is to bind to a single stranded DNA (to the lagging strand) near the replication fork and then move into the neighboring double stranded region. This forces the separation of the 2 strands, by disrupting hydrogen bonds.
   c) This function requires energy provided by ATP. **2ATP** molecules are consumed to separate each base pair.
   d) Once the strands separate, helix destabilizing proteins bind to them and preventing reformation of double helix DNA.
3. **Topoisomerase enzymes (I and II):** These are a group of enzymes that are responsible for forming the **"swivels"** between the separated and non separated 2 strands of double helix. This prevents formation of supertwisting and rotation of the entire chromosome ahead of replication fork.

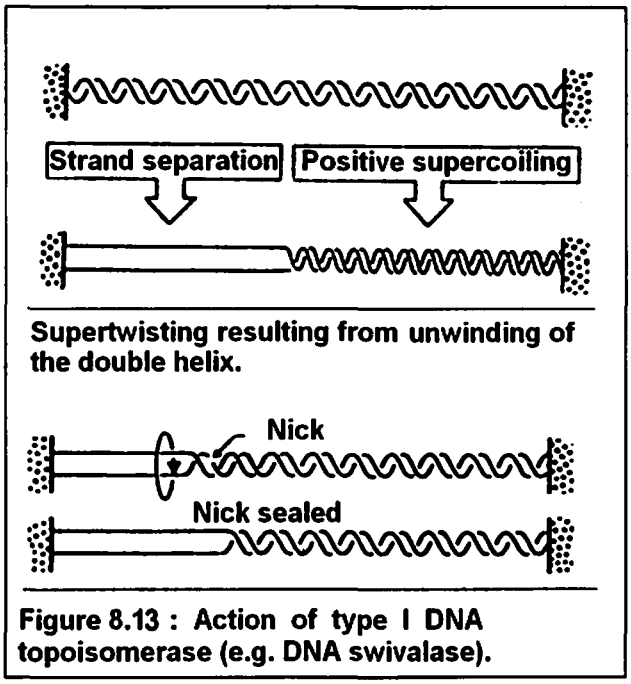


Figure 8.13 : Action of type I DNA topoisomerase (e.g. DNA swivalase).

Supertwisting makes further separation more difficult and rotation of the entire chromosome consumes a lot of energy.

Notes:

1) ***Rotation*** *can be demonstrated by tying the ends of two ropes together, twisting them to form a double helix, and then pulling the two ropes apart in the center of the helix.*

2) ***Supertwisting*** *can be demonstrated by holding the two ends of the previous rope in a fixed position and the two strands at the other ends are pulled apart (figur8.14).*

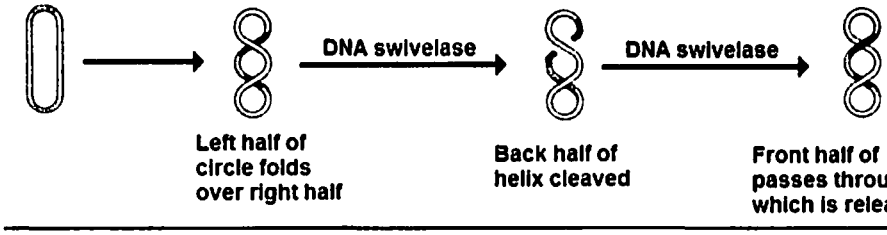


Figure 8.14: Action of type I topoisomerase (DNA swivelase). Oraby

a) **Topoisomerase I (DNA swivelase):**

1) **It cuts and rejoins a single strand of double helix.** This process does not require ATP as the energy released from the cleavage (cutting) of phosphodiester bond is reused to reseal (rejoin) the strand.

2) **By creating a transient "neck", the DNA helix on either** side of the nick is allowed to rotate at the phosphodiester bond **opposite** the nick, thus relieving accumulated supertwists (figure 8.14).

b) **Topoisomerase II (DNA gyrase):**

1) It binds tightly to both strands of DNA and make transient **breaks in both strands** of DNA helix to pass through the break and finally reseal the break. As a result, negative supertwists can be introduced that allow easier unwinding of the DNA double helix (figure 8.15).

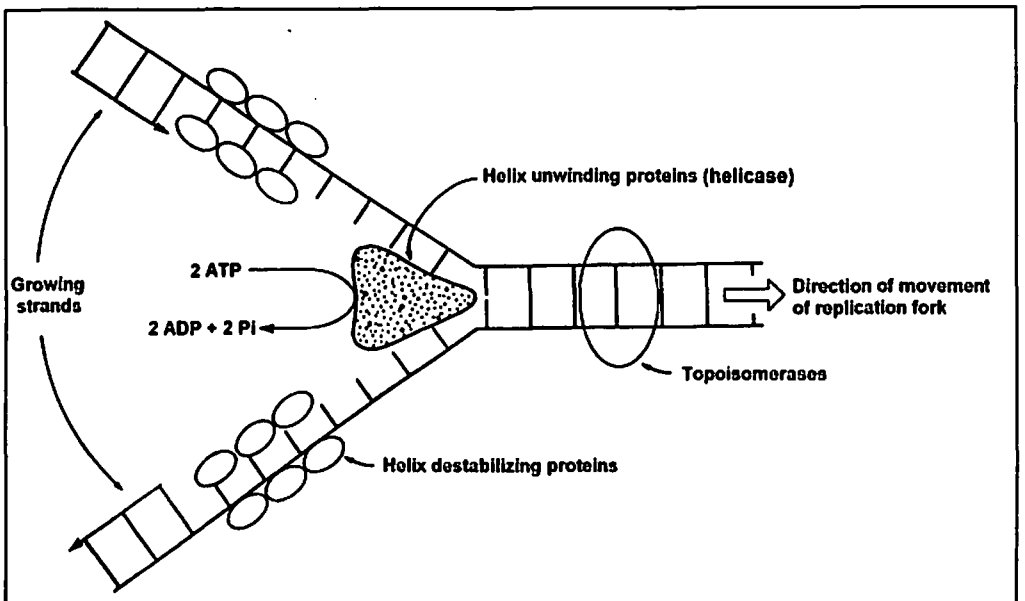


Figure 8.15: Proteins responsible for the separation of parental DNA.

III. *Steps of replication (DNA synthesis):*

A. Strand separation (unwiding of parental DNA).
B. Formation of RNA primers.
C. Synthesis of new DNA strands.
D. Excision of RNA primers and their replacement with DNA.

A. Strand separation (unwinding of parental DNA): This can be achieved by special group of proteins, which are:

1. Helix-destabilizing proteins (HD):

a) They keep the two strands of DNA separated in the area of replication.

b) They protect the DNA molecule from nuclease enzymes.

2. **Helix unwinding proteins (DNA helicase):**
   a) It binds to the single stranded DNA near the replication fork. It forces the separation of the 2 strands. 2 ATP molecules are consumed to separate each base pair.
3. **Topoisomerase enzymes I (swivelase) and II (gyrase):** Their function is to prevent:
   a) Supertwisting of DNA molecule which makes further separation more difficult.
   b) Rotation: of the entire chromosome that consume a lot of energy.

B. Formation of RNA primers:

1. Polymerase III is unable to assemble the first few nucleotides of a new strand by using the parent DNA strand as a template.
2. This assembly requires **RNA primer:** (figure 8.16)
   a) It is a short fragment of RNA, about 10 nucleotides in length.
   b) It is complementary and antiparallel to the DNA template.
   c) It has free -OH group at 3` end. This -OH serves as the acceptor of the first nucleotide from DNA polymerase III.
3. Synthesis of RNA primer requires **primosome,** which is a complex of an enzyme called: **RNA polymerase (primase)** and a **helicase** protein. Primosome binds with single stranded DNA and enables the initiation of synthesis of RNA primer.
4. RNA primer is later removed.

C. Synthesis of new DNA strands:

1. The substrates for DNA synthesis are 5`-deoxyribonucleotide triphosphates: **dATP, dGTP, dTTP and dCTP.**
2. Using the free 3`-OH group of the RNA primer as the acceptor of the first nucleotide, DNA polymerase III begins to add subsequent nucleotides (figure 8.17).
3. **Chain elongation:** as DNA polymerase III moves along the template strand, substrate nucleotides pair with the template according to the pairing rule i.e. A is paired with T and G is paired with C. Thus, the daughter strand will be complementary to the parent strand.
4. **DNA polymerase III can catalyze chain growth only in the 5` to 3` direction i.e. the new strand runs in 5` to 3` direction, while template strand runs in 3` to 5` direction (figure 8.16). Therefore the 2 daughter chains must grow in opposite directions, one towards replication fork (leading strand) and the other away from it (lagging strand).**

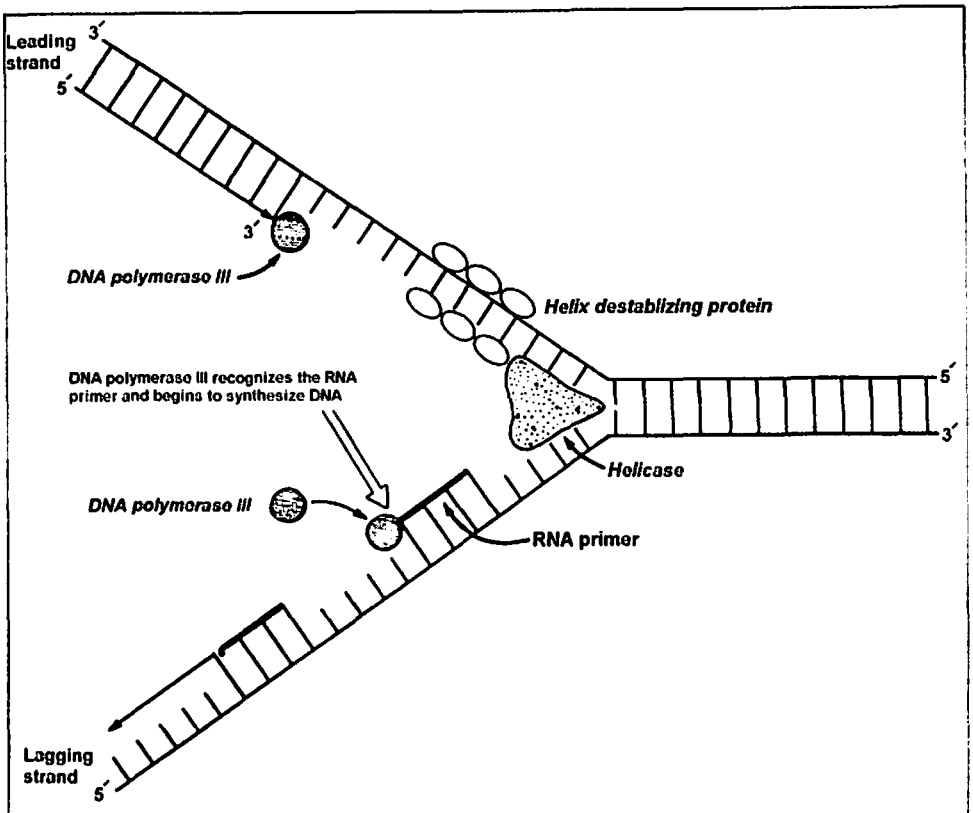


Figure 8.16: Elongation of leading and lagging strands.

5. The mechanism for synthesis of daughter strands is slightly different for each strand:
   a) **Leading strand:** is the strand that being copied in the direction towards replication fork. It is synthesized almost **continuously** (figure 8.16).
   b) **Lagging strand:** is the strand being copied in the direction away from the replication fork. It is synthesized **discontinuously** by forming small fragments of DNA called: **Okazaki fragments.** After formation of these fragments, they joined to become a single, continues strand. **Note** that RNA primers are common in lagging strand but they are few in leading strand.

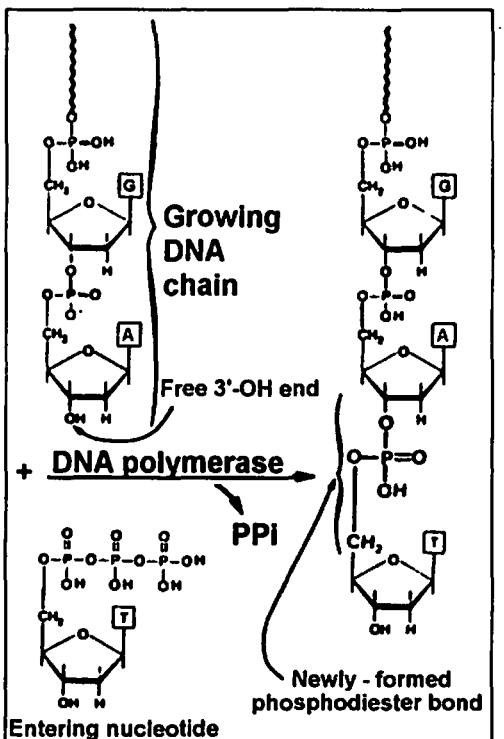


Figure 8.17: DNA synthesis: addition of a nucleotide to the 3'-OH end of the growing chain.

D. Excision of RNA primers and their replacement with DNA:

1. DNA polymerase III continues to synthesize DNA until it is blocked by a fragment of the RNA primer.
2. When this occurs, DNA polymerase I excises the RNA primer.
3. **DNA polymerase I fills gaps resulting from the excised RNA primers.**
4. The remaining nicks (spaces) are sealed by **DNA ligase:** The final phosphodiester linkage between 5` phosphate group on the DNA chain synthesized by DNA polymerase III and 3` hydroxyl group on the chain made by DNA polymerase I is catalyzed by **DNA ligase** (figure 8.18). The energy required for this joining is provided by cleavage of ATP to AMP and PPi.

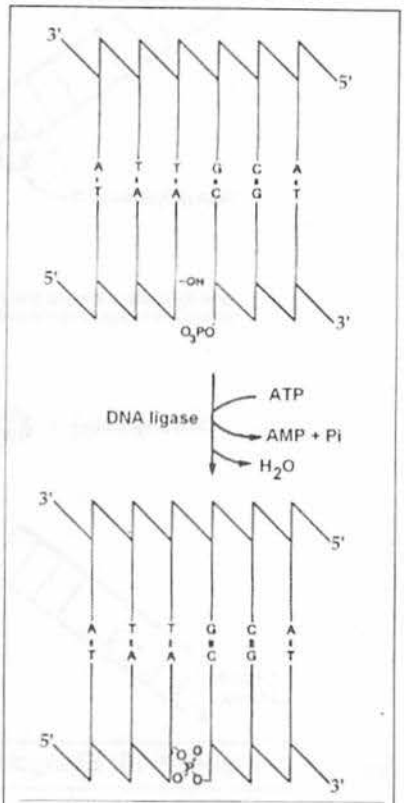


Figure 8.18: Formation of a a phosphdiester bond by DNA ligase

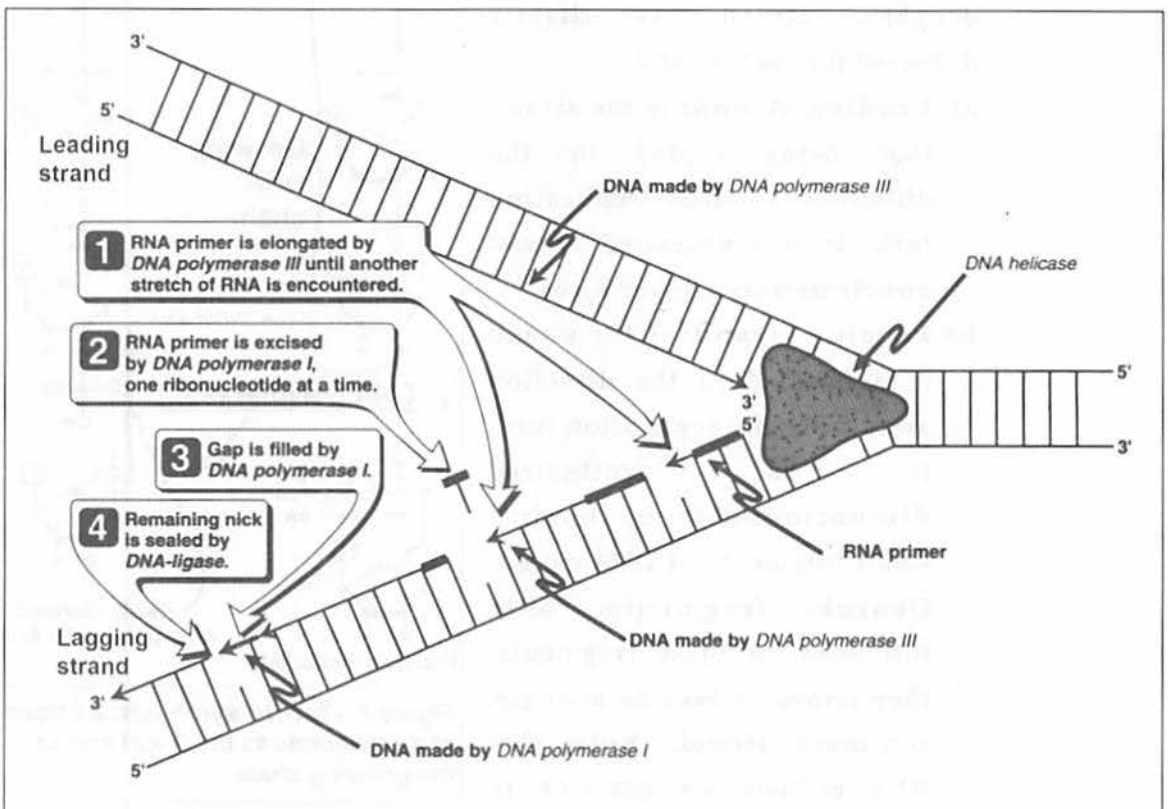
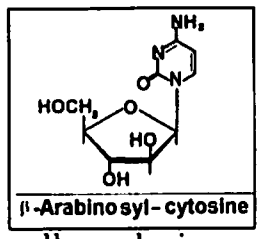


Figure 8.19: Removal of RNA primer and filling of the resulting "gaps" by *DNA polymerase I.*

IV.*Inhibition of replication:* DNA chain growth can be **blocked by certain nucleotide analogs,** which contain arabinose (arabinosylcytosine) instead of deoxyribose. This prevents further chain Elongation. These compounds slow the division of rapidly growing cells and viruses. Thus, they can be used as **anti-cancer chemotherapy.**



Summary of events of DNA replication (figure 8.19):

I-DNA replication is semiconsevative i.e. the daughter DNA consists of one strand from parent cell and the other is newly synthesized from free nucleotides present in the nucleus.

II-Steps:

A. ***Strands separation (unwinding of parental DNA):*** This can be achieved by special group of proteins:
1. **Helix-destabilizing proteins (HD):**
   a) They keep the two strands of DNA separated in the area of replication.
   b) They protect the DNA molecule from nuclease enzymes.
2. **Helix unwinding proteins (DNA helicase):**
   a) It binds to the single stranded DNA near the replication fork. It forces the separation of the 2 strands. 2 ATP molecules are consumed to separate each base pair.
3. **Topoisomerase enzymes I (swivelase) and II (gyrase):** Their function is to prevent:
   a) **Supertwisting of DNA** molecule which makes further separation more difficult.
   b) **Rotation:** of the entire chromosome that consume a lot of energy.

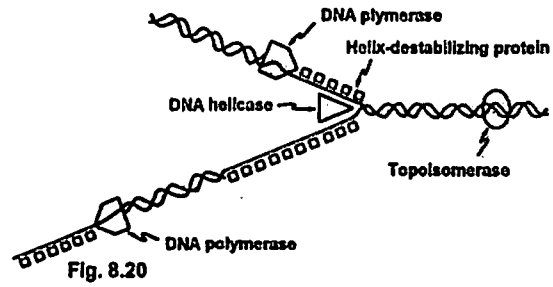


Fig. 8.20

B. ***Formation of RNA primer:*** It is short fragment of RNA synthesized under control of primosome which is a complex of RNA polymerase enzyme and helicase protein:
1. It is complementary and antiparallel to the DNA template.
2. It is common in lagging strand but they are few in leading strand.
3. It has free -OH group at 3` end which serves as the acceptor of the first nucleotide from DNA polymerase III.
4. RNA primers are later removed.

C. ***Synthesis of new DNA strand:***
1. The substrates for DNA synthesis are dATP, dGTP, dTTP, dCTP. As the DNA polymerase III moves along the template strand, different nucleotides are added according to the pairing rule i.e. adenine is paired with thymine and guanine is paired with cytosine. Thus, daughter strand will be complementary to the parent strand.
2. The chain growth occurs only in the 5` to 3` direction:
   a) The leading strand is synthesized almost continuously.

b) The lagging strand is synthesized discontinuously by forming small fragments called Okazaki fragments. After these fragments are formed, they joined to become a single continuous strand.

D. ***Excision of RNA primers and their replacement with DNA:***

1. DNA polymerase III continues to synthesize DNA until it is blocked by a fragment of the RNA primer.
2. When this occurs, the RNA primers are excised by DNA polymerase I.
3. Gaps resulting from the excised RNA primers are filled by DNA polymerase I.

4- The remaining nicks (spaces) are sealed by DNA ligase.

Summary of types and functions of enzymes responsible for DNA replication:

Prokaryotic	Eukaryotic	Catalytic activity
DNA polymerase I	DNA Polymerase α	*Remove RNA primers and fill the gaps that left (between Okazaki fragments) by synthesizing DNA. *DNA repair.
DNA polymerase II	DNA Polymerase ε	Proofreading and repair By removing all incorrect bases that may occur in the newly formed strand ➢ Identifying copying errors and correct them. ➢ It double checks each base pairing: * One before insertion of the nucleotide. * Later by follow up.
-	DNA polymerase β	DNA repair
-	DNA polymerase γ	Mitochondrial DNA synthesis.
DNA polymerase III	DNA polymerase δ	Synthesis of leading strand. Synthesis of Okazaki fragments.
Primosome (Primase+Helicase)	Primosome (Primase+Helicase)	Synthesis of RNA primers.
DNA Ligase	DNA Ligase	Seal the nicks (gaps) between the newly synthesized segments and the DNA strand.

Summary of types and functions of proteins responsible for DNA replication:

Prokaryotic	Eukaryotic	Catalytic activity
Enzymes: DNA polymerase I, II, III, primose and ligase	DNA polymerases α, ε, β, γ and δ, primosome and DNA ligase.	See the previous table
DNA Helicase	Same	It forces the **separation** of the 2 strands and prevents rejoining of them.
Helix destabilizing proteins (HD)	Same	They protect the DNA molecule from **nuclease** enzymes, and * They keep the 2 strands of DNA separated in the area of replication.
Topoisomerases	Same	* They prevent **super twisting** of DNA molecule which make further separation more difficult. * They prevent the **rotation** of the entire chromosomes that consumes a lot of energy.

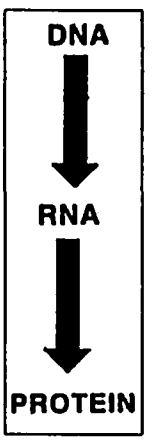
V. *Post-replication modifications of DNA:* By methylation:

A. Site of methylation. The site of DNA methylation in eukaryotes and prokaryotes is always on cytosine residues in C≡G base pairs.

B. Functions of methylation:

1. **In prokaryotes**: to prevent degradation of host DNA by restriction enzymes. The methylated host genome is resistant to the action of these enzymes. Viral DNA is not methylated and these enzymes can degrade it.
2. **In eukaryotes**: Methylation is important in DNA repair by a mechanism called: **mismatch repair** (see DNA repair).

VI. *Reverse transcriptase enzyme:* (also called RNA dependant DNA polymerase enzyme):



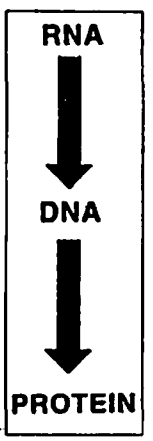
A. In cells, the flow of genetic information appears to be in one direction from DNA to RNA and from RNA to protein.

B. There are some species of viruses called **retroviruses.** The genetic material in these viruses is single stranded RNA and not DNA. They also contain an enzyme called reverse transcriptase.

C. Reverse transcriptase can reverse the first step of the flow of genetic information i.e. from RNA to DNA.

D. The produced DNA will undergo:

1. Replication to produce more viruses.
2. Entrance in the DNA of the host.



E. Steps of replication:

1. At first, reverse transcriptase synthesizes a RNA-DNA hybrid molecule using:
   a) RNA genome as a template.
   b) dATP, dTTP, dGTP and dCTP as substrates.
2. Then RNA strand is degraded by an enzyme called: **RNase H.**
3. The remaining DNA strand in turn serves as a template to form a double stranded DNA molecule containing the information originally present in the RNA genome of the virus.
4. The newly synthesized viral double stranded DNA enters the nucleus of the infected cell and can integrate by recombination into a host chromosome.

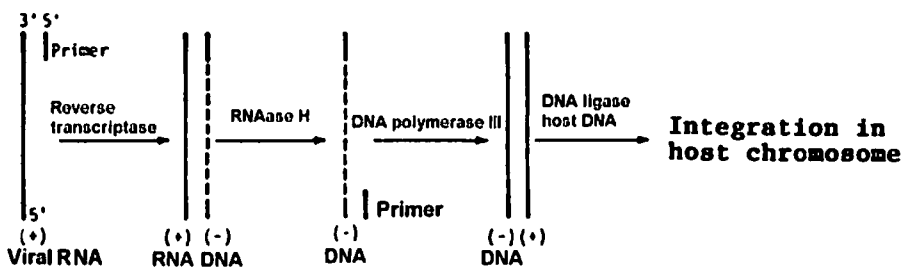


Figure 8.21:
Shows how double stranded DNA complementary to viral RNA is formed.

F. Examples of retroviruses are the HIV (AIDS) virus, hepatitis A virus and some tumor viruses.

G. Reverse transcriptase enzymes are important in **recombinant DNA technology.**

VII. *DNA repair*:

A. Definition:

1- **DNA repair is a mechanism to repair damaged DNA.**

2- Repair is important to maintain the genetic information and survival of a particular organism as well as the survival of the species.

3- If the damage is not repaired, a permanent mutation may be introduced that can result in serious diseases

B. Causes of DNA damage:

1. **Physical agents** e.g. X-ray, and electromagnetic waves as that produced from mobile phones.
2. **Chemical agents** e.g. Free radicals and some anti-malignant drugs.
3. **Ionizing radiation.**

C. Types of DNA damage:

1. **Single base alteration:**
   a) **Depurination** i.e. removal of a purine.
   b) **Deamination** of cytosine to uracil.
   c) **Deamination** of adenine to hypoxanthine.
   d) Insertion or deletion of nucleotide.
   e) Base analog incorporation.
2. **Two bases alteration:**
   - Formation of thymine-thymine dimer by ultraviolet light.
3. **Chain breaks:** e.g. phosphodiester bonds can be broken.
4. **Cross linkage:** Between bases in same or opposite strands.

D. Fate of damaged DNA: The damaged region of DNA may be:

1. **Repaired.**
2. **Replaced** by DNA recombination.
3. **Retained:** Retention leads to mutations and cell death.

Types of DNA repair:

1- Excision repair:
- a- Thymine-thymine dimer.
- b- Cytosine deamination to uracil.

2- Mismatch repair.

3- Double strands DNA break repair.

E. Mechanisms (types) of DNA repair:

1. **Excision repair:** It is used to repair many kinds of damage that involve only one strand e.g. thymine dimers and cytosine deamination to uracil.

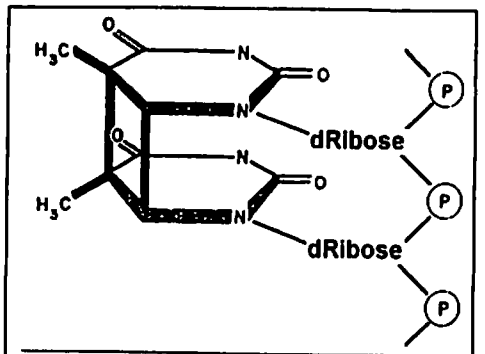


Figure 8.22: Thymine thymine dimer.

a) **Repair of thymine-thymine dimer:**

1) It results from covalent joining of 2 adjacent thymines.

2) It is caused by ultraviolet rays.

3) These thymine-thymine dimers prevent DNA polymerase from replicating the DNA strand beyond the site of dimer formation.

4) **The thymine-thymine dimer is excised and repaired as follows:**

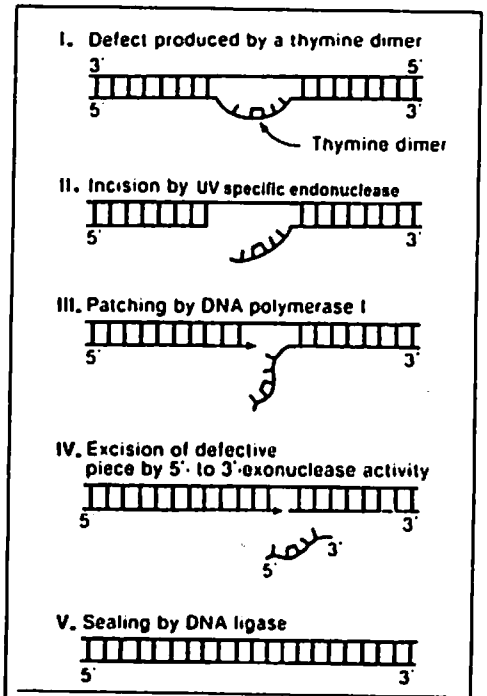


Figure 8.23: Excision of a thymine-thymine dimer.

i- First, **a UV-specific endonuclease** recognizes the dimer and **makes** a nick (cut) near it, usually at the 5` end.

ii- DNA polymerase I fills the gap resulting from the cut in the direction of 5` to 3`. The other strand of DNA acts as a template.

iii- The thymine dimer region is excised by the 5` exonuclease activity of DNA polymerase I.

iv-Finally, DNA ligase seals the gap between the newly synthesized segment and the main strand (figure 8.23).

Xeroderma pigmentosum:

- *This is a **malignant** disease.*
- *It is an autosomal recessive disease,*
- ***Cause:** Absence of UV specific endonuclease required for the recognition of the dimmer.*
- ***Signs and symptoms:** Individuals affected by this disease are sensitive to UV light which causes extensive accumulation of thymine-thymine dimers in skin cells with malignant transformation.*

b) **Repair of cytosine deamination to uracil:**

1) Abnormal base, uracil (U) is recognized by **specific glycosylase** that cleaves the uracil from the strand.
2) Another enzyme, endonuclease cuts the phosphodiester backbone on the 5` side.
3) DNA polymerase I then fills the gap with the correct cytosine (C) base.
4) DNA ligase seals the break (figure 8.24).

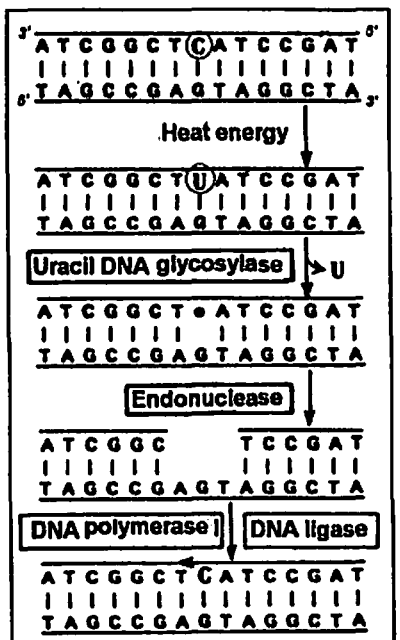


Figure 8.24: Repair of cytosine deamination to uracil.

2. Mismatch repair:

a) Mismatch error means: (C is paired with A rather than G) or (G is paired with T rather than C). The presence of unpaired bases is also a mismatch error.

b) **The mechanism of repair:**

1) The correct strand is **methylated** and the newly synthesized is not. This difference allows the repair enzymes **to identify** the strand that contains nucleotide errors, which require replacement.
2) The defective region is recognized by **GATC endonuclease** that makes a single-strand cut adjacent to methylated GATC sequence.

3) The defective DNA strand is removed by exonuclease, and repaired by polymerase I according to base pairing rule.

4) The nick is then sealed by ligase.

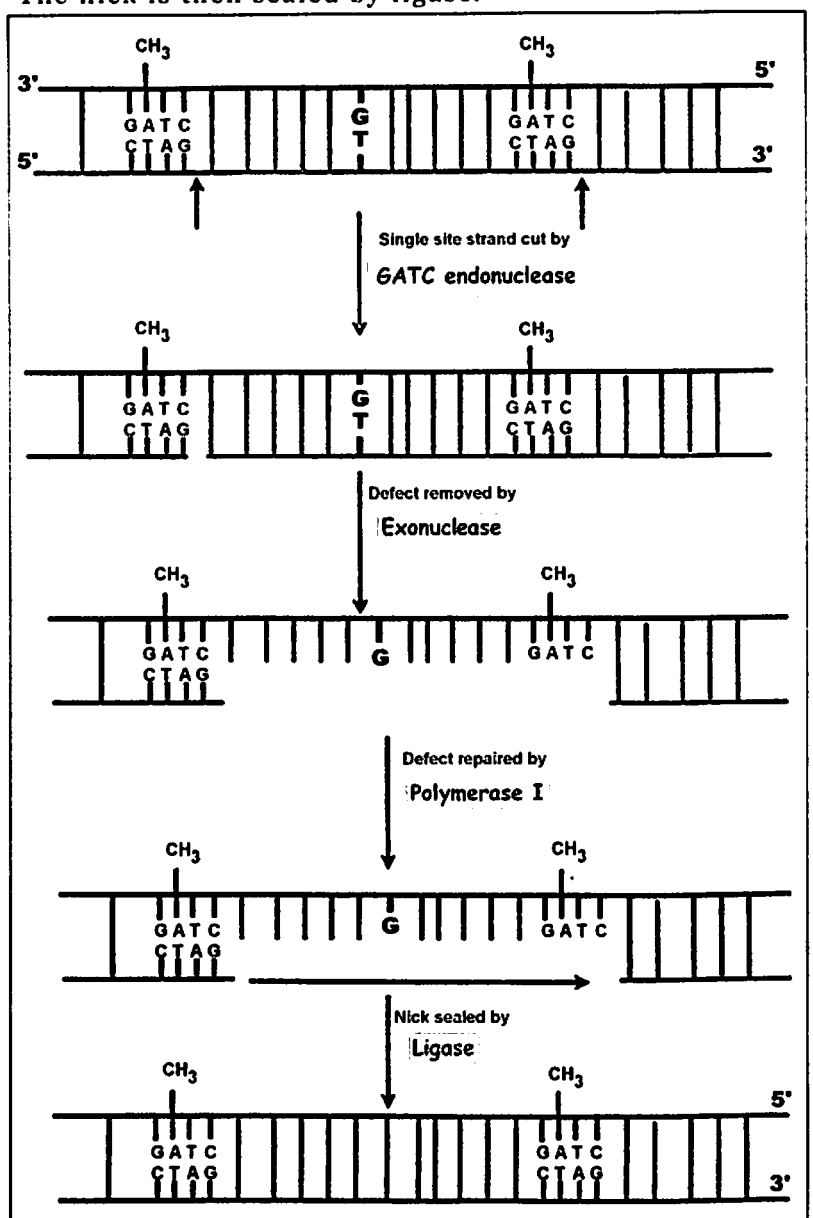


Fig. 8.25: Mechanism of Mismatch repair. *Oraby*

3. Double strand (ds) DNA break repair:

a) It is an important mechanism for repairing damaged DNA as a result of ionizing radiation, free radicals, and chemotherapy.

b) **Two proteins are involved in the joining of ds break, these are:**

1) **Ku protein:** This protein can bind the free DNA ends and it has also a latent ATP-dependant **helicase** activity.

2) **DNA-PK** (=DNA-dependant protein kinase): It has binding site for DNA free ends and another for ds DNA just inside the break.

c) **Mechanism of repair:**

1) **Ku and DNA-PK** combine to the free ends of ds break of DNA.
2) They **approximate** the 2 ends of the break.
3) They **unwind** the free ends by helicase like activity of **Ku**.
4) They aligned fragments to form **base pairs.**
5) The extra-ends are **removed** by exonuclease.
6) The **gap** is filled and sealed by DNA ligase.

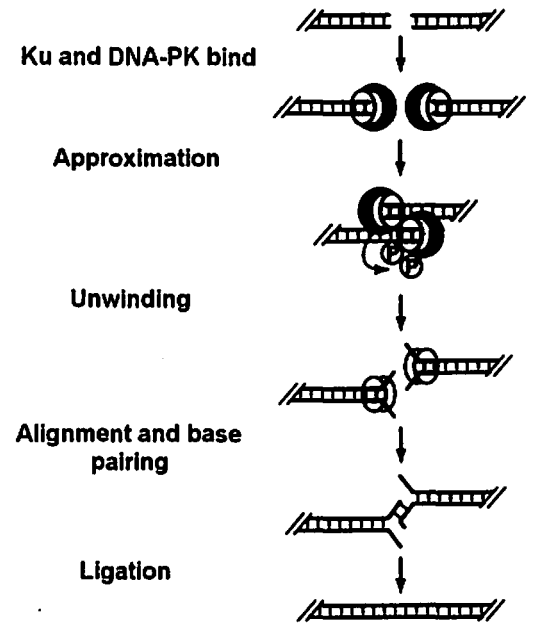


Figure 8.26: Double strand break repair of DNA.

Summary of DNA repair:

Mechanism	DNA error	Repair
Excision repair	Thymine-thymine dimer (Pyrimidine-pyrimidine dimer)	•single cut by **UV specific endonuclease.** •Gap filled by DNA polymerase I. •Removal of thymine-thymine dimer region by exonuclease. •Remaining nick is sealed by ligase.
	Cytosine deamination to uracil (C → U + NH2)	•Removal of abnormal base (U) by uracil **DNA glycosylase.** •Removal of the remaining phosphodiester backbone by endonuclease. •Gap is filled by DNA polymerase I. •Remaining nick is sealed by ligase.
Mismatch repair	• Single base error G=U instead of G≡C • 2-5 unpaired bases	•**Methylation** of correct strand near error at (GATC). •Single cut → by **GATC endonuclease** •Removal of error by exonuclease. •Repair of defect by polymerase I •Remaining nick is sealed by ligase.
Double stranded DNA break repair	Complete cut of the double stranded DNA	•Binding of **Ku and DNA-PK** proteins to both ends of cut. •Approximation of both ends. •Unwinding of free ends by helicase activity of Ku protein. •Alignment and base pairing of free ends. •Excision of extra nucleotide tails by exonuclease. •Gaps are filled and sealed by ligase.

VIII. *Regulation of DNA synthesis(cell cycle)*

A. In animal cells, including human cells the replication of DNA genome occurs only at specific time during the life span of the cell. This period is called the **synthesis or S phase.**

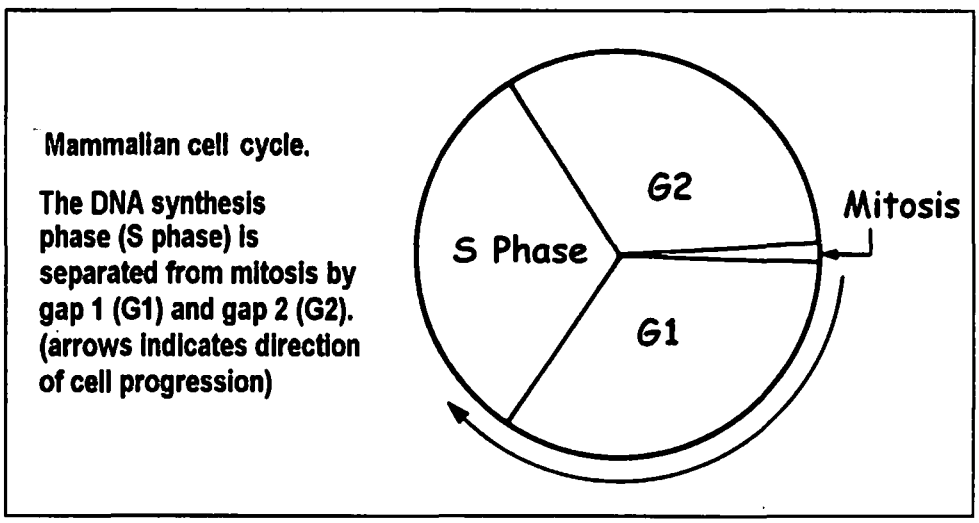
B. The S phase is usually separated from the mitotic phase by non-synthetic periods called **gap 1 (G1) and gap 2 (G2)** occurring before and after S phase respectively.

C. In eukaryotic cells, there is a gene that produces protein called **cyclin.** Such protein regulates the transition from one phase of the cell cycle to another.

D. **<u>The cell regulates its DNA synthesis</u>** by allowing it to occur only at specific time i.e. S phase. During this time:

1. Cell contains excess quantities of DNA polymerase than that during the non synthetic phases of the cell cycle.
2. There is excess activity of enzymes that is responsible for the formation of the substrates for DNA synthesis i.e. enzymes for formation of dATP, dTTP, dGTP and dCTP.

E. During S phase, the nuclear DNA is completely replicated once and only once.



Mammalian cell cycle.

The DNA synthesis phase (S phase) is separated from mitosis by gap 1 (G1) and gap 2 (G2). (arrows indicates direction of cell progression)

RNA synthesis = Transcription

I. **Definition:** Transcription is the process of the RNA synthesis.

II. **Requirements for RNA synthesis:**

A. ***Transcription unit*** *(promotor region, transcribed region, termination region and enhancers)*
B. ***Four ribonucleotide triphosphates:*** *ATP, GTP, UTP and CTP*
C. ***RNA polymerase enzymes***

A. **Transcription unit:** It is the stretch (part) of DNA, which includes: promotor region, transcribed region, termination region and enhancers. Transcription occurs on one of the 2 strands of the DNA (=template strand) and never on both complementary strands.

1. **Promotor region:** This is certain sequence of bases located at the beginning of stretch. It is important for initiation of transcription to occur and can be recognized by RNA polymerase.

a) **Prokaryotic promotor:** It is composed of:

1) **The pribnow box:** Which is stretch of 6 nucleotides (TATAAT) located about 10 bases to the left of the transcription initiation site.
2) A second nucleotide stretch **(TGTTGACA)** that located about 35 bases to the left of the transcription initiation site.
3) 19 Bases (nucleotides) in between two stretches.
4) Both pribnow box and TGTTGACA are regions that can be recognized by RNA polymerase.

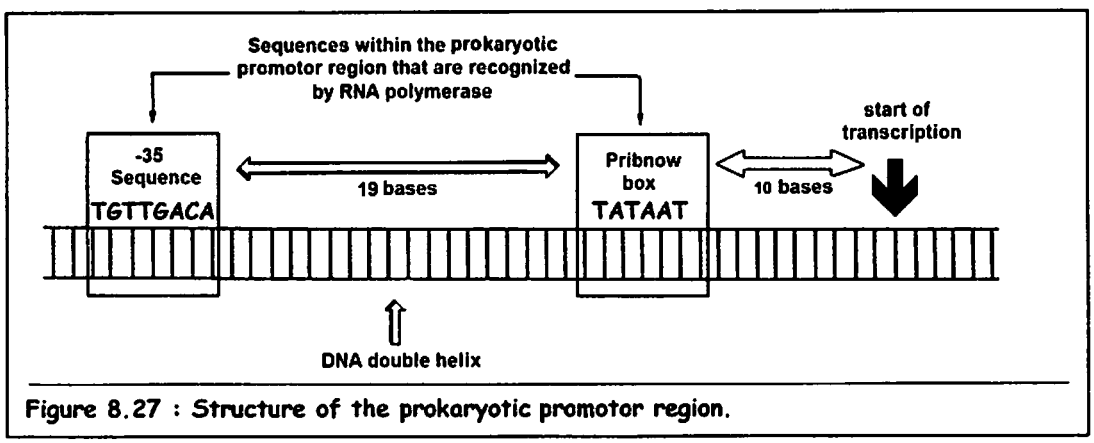


Figure 8.27 : Structure of the prokaryotic promotor region.

b) **Eukaryotic promotor:** It is composed of:

1) **The Hogness or TATA box:** Which is stretch of nucleotides that almost identical to that of pribnow in prokaryotes. It is located

almost 25 nucleotides to the left of the transcription initiation site.

2) **CAAT box:** Which is located about 70 to 80 nucleotides to the left of the transcription initiation site.

3) **40 bases** between the two stretches.

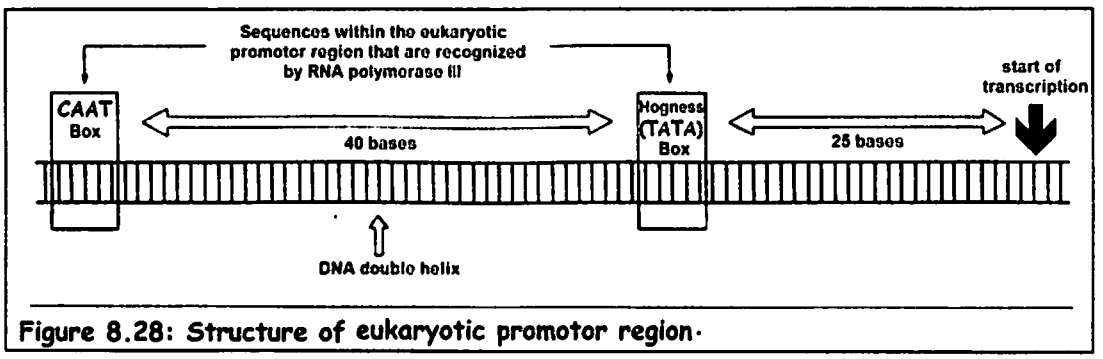
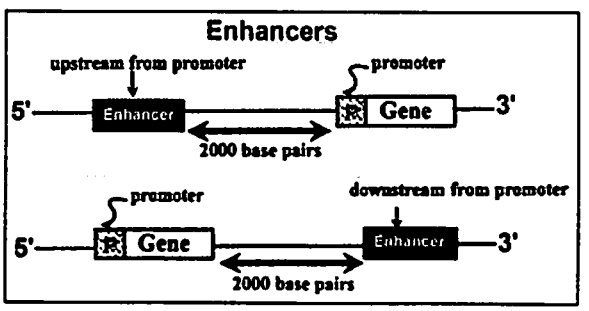


Figure 8.28: Structure of eukaryotic promotor region.

2. **Transcribed region:** Which is stretch of DNA that is to be transcribed into RNA molecules.
3. **Termination region:** Which is a stretch of DNA located at the end of transcribed DNA.
4. **Enhancers:**



a) These are specific segments present in DNA, which control and increase the rate of transcription in eukaryotes.

b) Its position is several thousands base pairs apart from the transcription unit.

c) It may be upstream (before promotor) or downstream (after termination region).

d) When a specific protein or steroid hormones are attached to enhancer, the rate of transcription will increase.

B. Four ribonucleotide triphosphates: ATP, GTP, UTP and CTP.

C. RNA polymerase enzyme; RNAP (=DNA-dependant RNA polymerase):

1. **Prokaryotic RNA polymerase:**

a) In prokaryotes e.g. E.coli bacterium, RNA polymerase **(RNAP)** enzyme synthesizes all types of RNA molecules on DNA template.

b) The holoenzyme of the RNA polymerase consists of a core molecule and specific protein factor (sigma [σ] factor) figure 8.29.

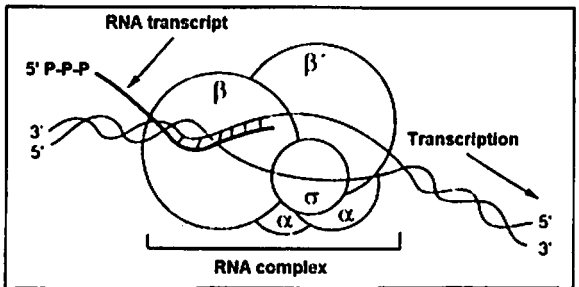


Figure 8.29: RNA polymerase in prokaryotes.

1) **The core molecule:**
consists of 4 subunit; 2 of them are identical (the alpha subunits). The other 2 (β and β' subunits) are similar in size but not identical. In addition, RNAP contains 2 zinc molecules.

2) **The sigma factor [σ]:** enables the polymerase to recognize promotor regions on DNA. It also helps the core enzyme to attach more tightly to the promotor site.

c) The RNA polymerase itself may recognize some regions on DNA that signal the termination of transcription.

2. <u>Eukaryotic RNA polymerase</u>: There are three species of RNA polymerase in eukaryotic cells:
   a) **RNA polymerase I:** Which synthesizes the large ribosomal RNAs in the nucleolus.
   b) **RNA polymerase II:** Which synthesizes messenger RNA and recognizes the promotor region.
   c) **RNA polymerase III:** Which synthesizes the small RNAs, including the tRNA and the small ribosomal RNA.

III. *Steps in RNA synthesis:* (initiation, elongation and termination):

A. <u>Initiation</u>:

1. At first, RNA polymerase holoenzyme binds with the promotor area:
   a) The sigma [σ] subunit enables polymerase to recognize promotor region on DNA.
   b) The β` subunit binds to the DNA template.
   c) The β subunit binds to the nucleotide substrates.
2. Binding of RNA polymerase to DNA template leads to local separation (unwinding) of the DNA double helix into **template** and **coding** strands.

(template strand of the gene is that part of DNA that is transcribed or copied into an RNA molecule while the other DNA strand is called coding strand of the gene).

3. The first nucleotide of RNA transcript at the initiation site is almost always purine.

B. Elongation:

1. At the template strand, formation of RNA molecule begins at the 5`end by core enzyme with the release of sigma factor.
2. Then elongation of the RNA molecule occurs from 5` to 3` end, antiparallel to its template.
3. The nucleotide building blocks are 5` ribonucleoside triphosphates (ATP, GTP, CTP and UTP). They are inserted in RNA molecule according to the pairing rule i.e. A-U, G-C, T-A and C-G. Pyrophosphate (PPi) is released when each new nucleotide is added to the growing chain **e.g. GTP→GMP + PPi**
4. RNA polymerase forms a phosphodiester bond between the 3`OH of one ribose sugar and 5`OH of the next ribose.
5. The process of elongation of RNA chain continues until a termination region is reached.

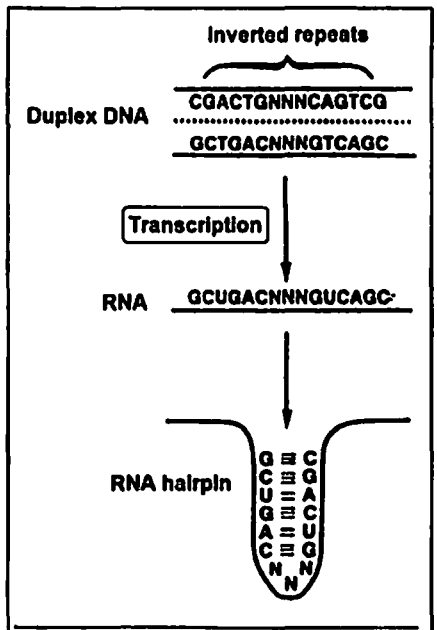


Figure 8.30: An example of palindrome.

C. Termination: Termination region on DNA template can be recognized either by:

1. **Rho [ρ] factor,** which may be required for the release of both RNA strand and RNA polymerase (rho dependant termination).
   - when RNA polymerase enzyme reaches the termination site, rho factor binds with it causing termination (figure 8.31).
2. **RNA polymerase enzyme itself** (rho independent termination).
   - When RNA polymerase enzyme reaches the termination site it may meet a DNA palindrome that causing slowing down of RNA polymerase and termination of transcription occurs.

Note

- **Palindrome** is a region of a double stranded DNA in which each of the two strands has the same sequence when read in the same direction e.g. in the 5` to 3` direction.
- The RNA transcript of the DNA palindrome can form a stable **hairpin** structure, which is a self-complementary structure. This hairpin structure causes slowing down of RNA polymerase at the termination site (figure 8.30).

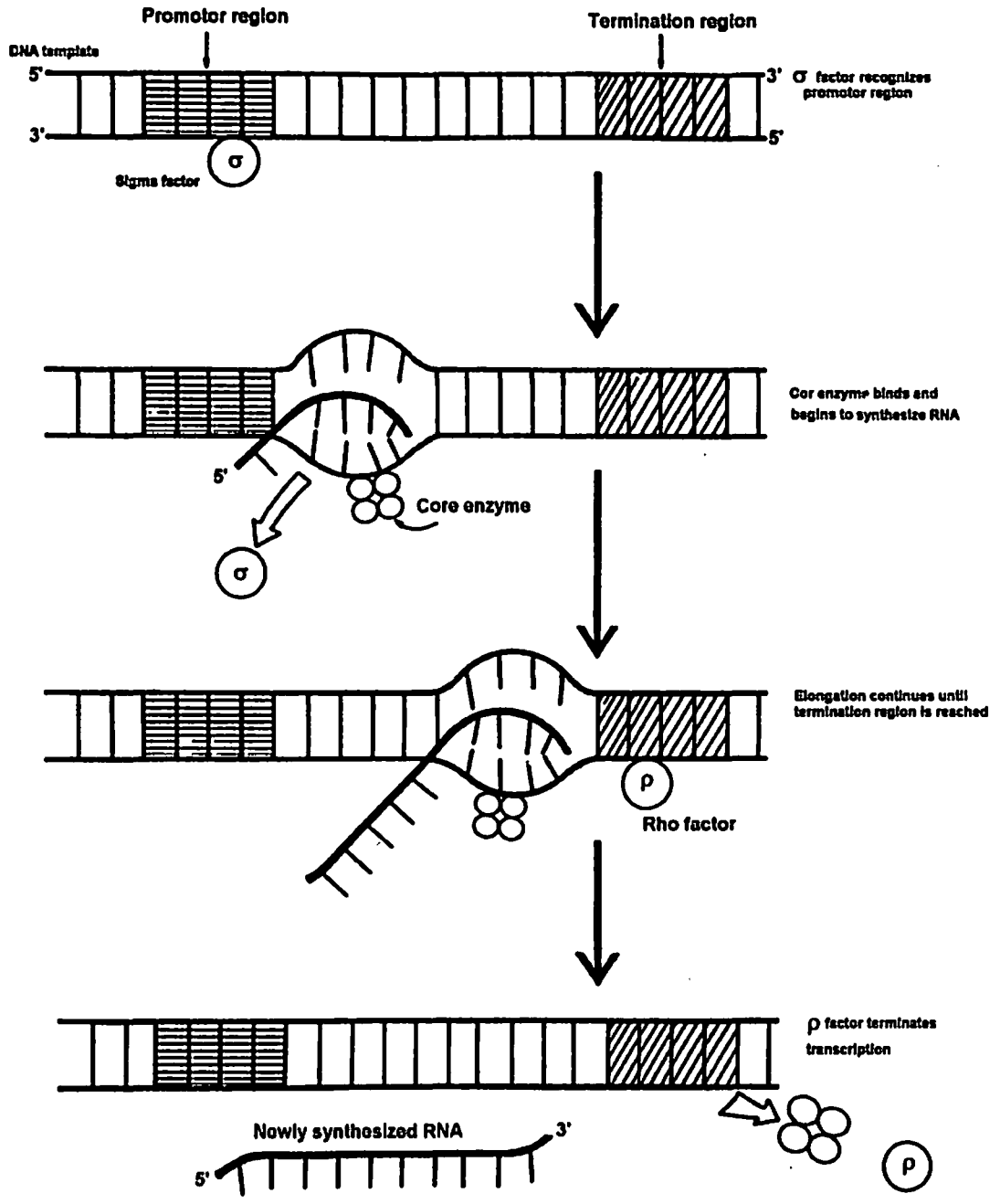


Figure 8.31: Steps in RNA synthesis (transcription).

IV. *Inhibition of transcription by antibiotics:*

A. **Rifamycin** is an antibiotic, which binds to the B subunit of RNA polymerase, preventing the incoming nucleotide from binding to the initiation site.

B. **Actinomycin D** is an antibiotic, which binds to the DNA template and prevents the movement of RNA polymerase along the DNA.

V. *Posttranscriptional modification of RNA*: These are changes which occur to RNA after transcription:

A. **Eukaryotic messenger RNA:** primary mRNA is called heterogeneous nuclear RNA **(hnRNA)**. HnRNA is modified into mature mRNA in the nucleus by capping, tailing and splicing:

1. **5`-capping:** The 5` end of the mRNA requires a cap which is 7 methyl-guanosine triphosphate. It is attached by a 5` to 5` triphosphate linkage (figure 8.32).
   a) This reaction needs an enzyme called: **guanyl transferase.**
   b) The function of this cap is to facilitate the initiation of translation, and protects the 5` end of mRNA from attack by 5` exonucleases.

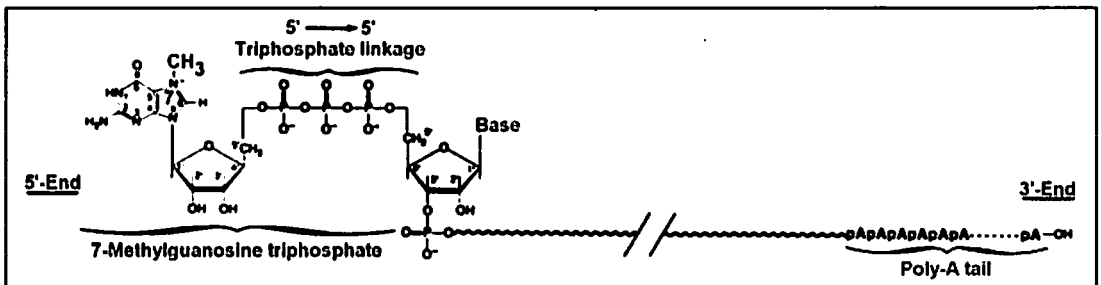


Figure 8.32: Post-transcriptional modifications of mRNA showing the 7-methylguanosine cap and poly (A) tail.

2. **Addition of a poly (A) tail:** Most mRNAs require almost 40 to 200 adenine nucleotides added at 3` terminus to form a poly adenine (polyA) tail (figure 8.32).
   a) This reaction needs an enzyme called: **poly A polymerase enzyme.**
   b) The function of this tail is to protect the 3`end of mRNA from 3` exonuclease attack.
3. **Splicing (removal of introns):**
   a) hnRNA is formed of many pieces, some of them **(exons)** will be translated into amino acids. Others **(introns)** will not be translated into amino acids and must be removed before translation takes place (figure 8.33).

b) **Spliceosomes** are structures responsible for removal of introns from the hnRNA, and splicing **(ligation)** of both ends of exons to form mature mRNA containg only exons.

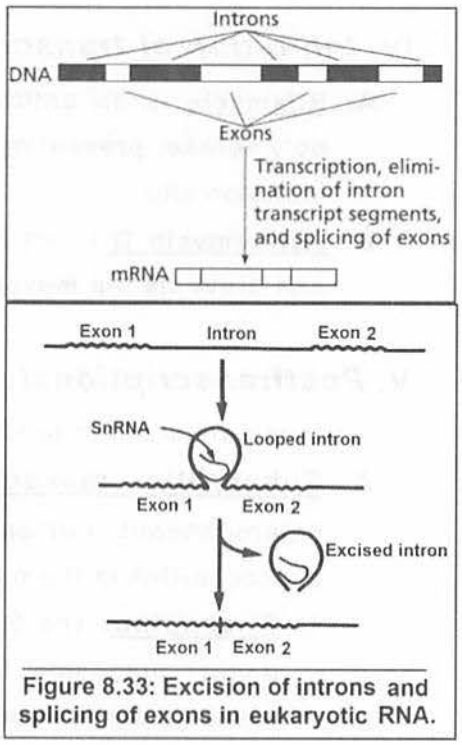


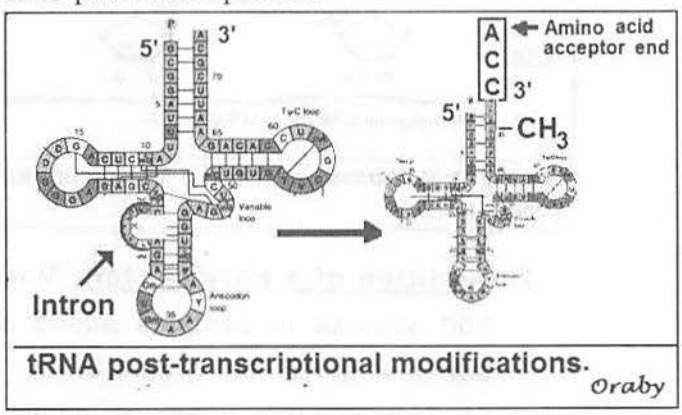
Figure 8.33: Excision of introns and splicing of exons in eukaryotic RNA.

1) **Spliceosomes consist of:**
   - i- The primary hnRNA.
   - ii- More than 50 proteins.
   - iii- Five Small nuclear RNAs (U1, U2, U5 and U4/U6). The role of small nuclear RNAs (snRNP) is to bind each end of the introns by forming base pair with each other.

2) Spliceosomes facilitate also the transport of mature mRNA from the nucleus to the cytoplasm.

B. Transfer RNA (tRNA):

1. The tRNA molecules serve as adaptor molecules for the translation of mRNA into protein sequence.
2. **Primary tRNA transcript are subjected to many modifications:**



tRNA post-transcriptional modifications. Oraby

a) **Reduction in size:** tRNA molecules are transcribed as **large precursors.** These precursors are **reduced in size** by specific class of ribonucleases.

b) **Attachment** of the characteristic **C.C.A. terminus** at the 3`end of the molecule.

c) **Methylation of** some bases of the tRNAs.

d) **Removal of a single intron** (10-40 nucleotides long), which present near to the anticodon loop. These introns

must be removed with splicing of exons to produce an active tRNA for protein synthesis.

C. Ribosomal RNA (rRNA):

1. In mammalian cells, rRNA is transcribed as a **single large precursor** molecule called **45S**, figure 8.34.
2. In the nucleus, 45S is methylated and cleaved by specific exonucleases and endonucleases to give 4 kinds of rRNA: **5S** rRNA, **5.8S** rRNA, **18S** rRNA and **28S** rRNA.

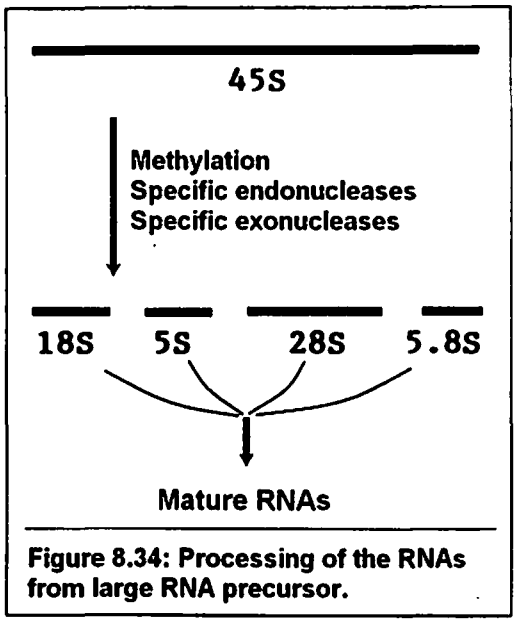


Figure 8.34: Processing of the RNAs from large RNA precursor.

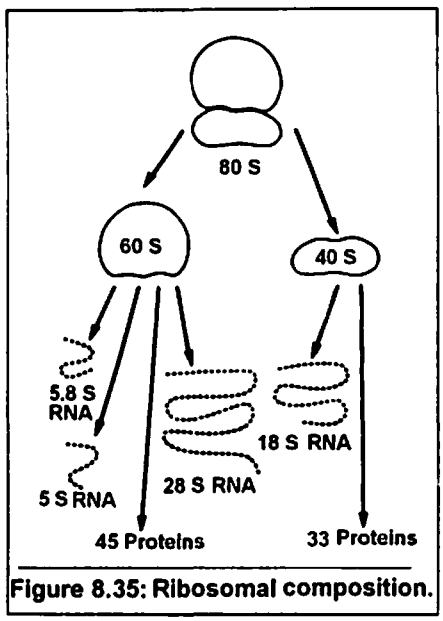


Figure 8.35: Ribosomal composition.

3. The 4 kinds of rRNA combine with a number of proteins to form ribosomes:
   - *a)* **Ribosomes:** are cytoplasmic nucleoproteins composed of 4 rRNA plus a number of proteins (figure 8.35).
   - *b)* These rRNA and proteins are distributed specifically between the two smaller and larger ribosomal subunits:
     1) **The smaller subunit is called 40S.** It contains one 18S rRNA and 33 proteins.
     2) **The larger subunit is called 60S.** It contains the remaining rRNAs (28S , 5.8S and 5S) and 45 proteins.
     3) Both smaller (40S) and larger (60S) subunits form the whole **80S ribosome.**
   - *c)* **Functions of ribosomes:** They are the site of protein synthesis within the cells. They contain enzymes essential for this synthesis.

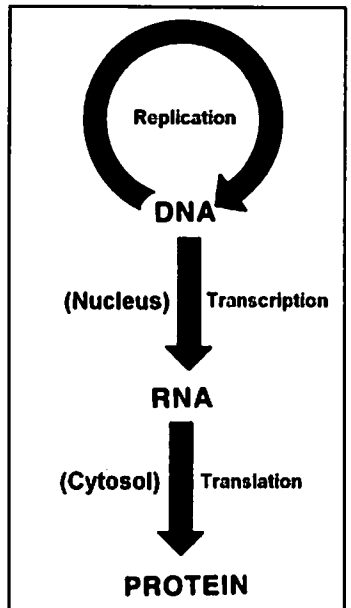
Translation of mRNA = protein synthesis

I. Introduction:

A. The genetic information of eukaryotes is contained in the sequence of purines (A&G) and pyrimidines (T&C) that constitutes the DNA in the nucleus. These bases are organized into 3 letter code words called **codons.** The collection of these codons makes up the genetic code, which is translated into a sequence of amino acids in the cytoplasm to form a polypeptide or protein.

B. DNA is not directly used as a template for protein synthesis. Instead a temporary RNA copy is made for each part of DNA (gene) that is to be expressed. This copy then directs the synthesis of protein. These two stages of gene expression are called transcription (discussed before) and translation respectively. Also this flow of information from DNA to RNA to protein is termed **"central dogma"**. It is descriptive of all organisms.

C. It is impossible to understand protein synthesis or explain mutations before the genetic code is explained.



The central dogma

II. Genetic code:

A. <u>The genetic code</u>:

1. **It is the sequence of nucleotides along the DNA that can be translated into the amino acids of proteins.** (= is the relationship between the sequence of bases in DNA and a sequence of amino acids in protein).
2. The genetic code is made up of a collection of codons.

B. <u>Codons</u>: are the individual words in the genetic code dictionary. Each codon is composed of 3 nucleotide bases.

> ***Definition of the codon:***
> *It is the sequence of three nucleotide bases on mRNA which determines the type and the position of the amino acid that will enter in the structure of protein molecule.*

1. The sequence of bases in codon is always read from its 5` to its 3` end by **adaptor molecules** (tRNA) that carrying specific amino acids.

2. There are 4 nucleotide bases: adenine (A), guanine (G), cytosine (C) and uracil (U) that can be used to produce the 3-base codons. Therefore there are 64 different combinations of bases, taken 3 at a time as shown in table 8.1. This table (or dictionary) can be used to translate any codon sequence and thus to determine which amino acids are coded for by the mRNA sequence. For example , the codon **5`-CAU-3`** codes for **histidine,** while **5`-AUG-3`** codes for **methionine.**
3. **Nonsense (termination) codons:** There are 3 codons, **UAG, UGA and UAA** do not code for amino acids and are called nonsense codons. These are termination codons as when one of them appears in a mRNA sequence, it indicates that synthesis of the peptide chain coded by that mRNA has been completed.

First base of codon	Second base of codon: U	Second base of codon: C	Second base of codon: A	Second base of codon: G	Third base of codon
U	UUU, UUC Phe; UUA, UUG Leu	UCU, UCC, UCA, UCG Ser	UAU, UAC Tyr; UAA, UAG Term.*	UGU, UGC Cys; UGA Term.*; UGG Try	U C A G
C	CUU, CUC, CUA, CUG Leu	CCU, CCC, CCA, CCG Pro	CAU, CAC His; CAA, CAG Gln	CGU, CGC, CGA, CAG Arg	U C A G
A	AUU, AUC, AUA Ileu; AUG Met + Init.†	ACU, ACC, ACA, ACG Thr	AAU, AAC Asn; AAA, AAG Lys	AGU, AGC Ser; AGA, AGG Arg	U C A G
G	GUU, GUC, GUA Val; GUG Val + Init.†	GCU, GCC, GCA, GCG Ala	GAU, GAC Asp; GAA, GAG Glu	GGU, GGC, GGA, GGG Gly	U C A G

Table 8.1: 64 different codons.

C. Characteristics of the genetic code (codons):

1. Specificity (unambiguous):

Each specific codon always codes for only a single same amino acid.

The genetic code is specific e.g. UUU codes only for phenylalanine and not for any other amino acid.

2. Degeneracy of the genetic code:

More than one codon can code for one amino acid.

There are 20 amino acids and 64 codons available. This indicates that multiple codons must code for the same amino acid. For example, arginine is coded by six different codons. Codons specifying the same amino acid are called **degenerate or synonyms.**

3. **Universality:**

The genetic code is universal. It is the same for all species of plants and animals.

with the exception of the mitochondrial genome where **AUA** codes for methionine instead of isoleucine and **UGA** codes for tryptophan instead of acting as termination codon.

4. **Non-overlapping:**

The genetic code is non-overlapping and commaless.

This means that the codons do not overlap each other.

Any code is read from a fixed starting point as a continuous sequence of bases, taken 3 at a time, without comma or punctuation between codons. For example, ABCDEFGHIJKL...is read as ABC / DEF / GHI / JKL / ...without any interruption between the codons. **Note that** if one nucleotide is either deleted from or added to the interior of the nucleotide sequence, the reading frame will be altered, and the resulting amino acid sequence may become radically different from this point onward, figure 8.36.

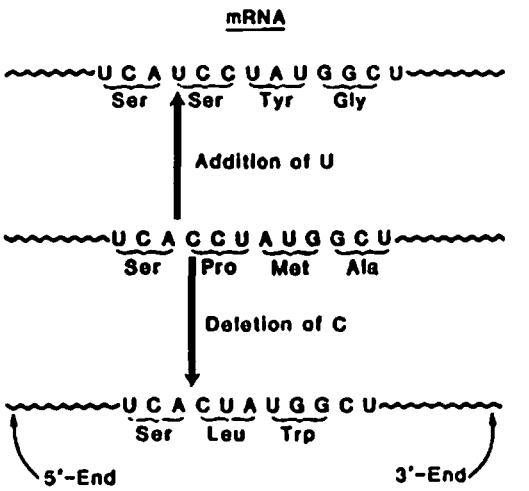


Figure 8.36: Addition or deletion of a single nucleotide can cause alteration in reading frame of mRNA.

D. Codon-anticodon recognition:

1. **Role of the anticodon sequence:** (figure 8.37):
   - *a)* Recognition of a particular codon on the mRNA sequence is accomplished by the anticodon sequence of the tRNA.
   - *b)* The binding of tRNA anticodon to the mRNA codon follows the rules of **complementary, antiparallel** binding. This means that the mRNA codon is read 5`→ 3` by an anticodon pairing which read in 3`→ 5` direction.

Note: when writing the sequence of **both codons and anticodons,** the nucleotide sequence must **always** be listed in the **5`→ 3` order.**

2. **The wobble hypothesis:** (figure 8.38):
   a) Some tRNAs recognize more than one codon for a given amino acid.
   b) The base at the 5` end of the anticodon (the first base of the anticodon) has sometimes the ability to pair with more than one type of bases of the codon (third base), as follows:

First base in the anticodon	Third base in the codon
A (Adenine) ➔	U (Regular)
C (Cytosine) ➔	G (Regular)
U (Uracil) ➔	G or A
G (Guanine) ➔	U or C
I (Inositol) ➔	A , C or U

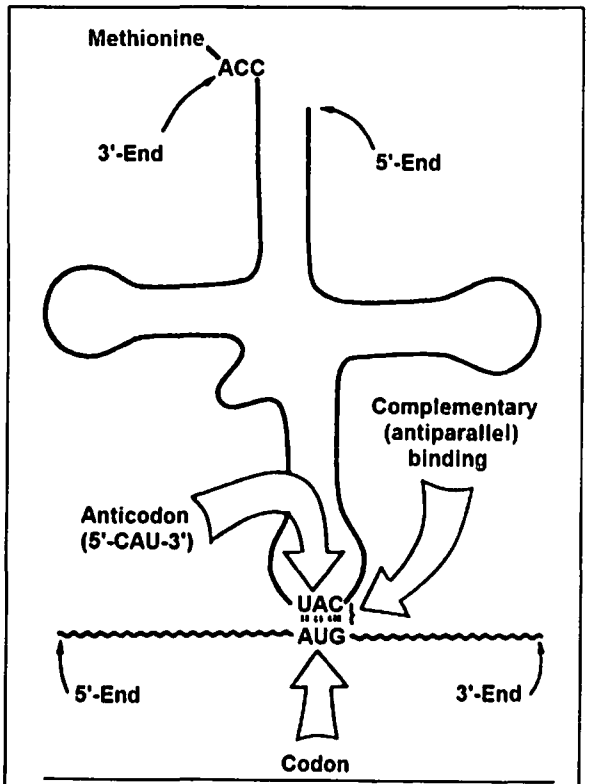


Figure 8.37: Complementary, antiparallel binding of the anticodon for methionyl - tRNA (CAU) to the mRNA codon for methionine (AUG)

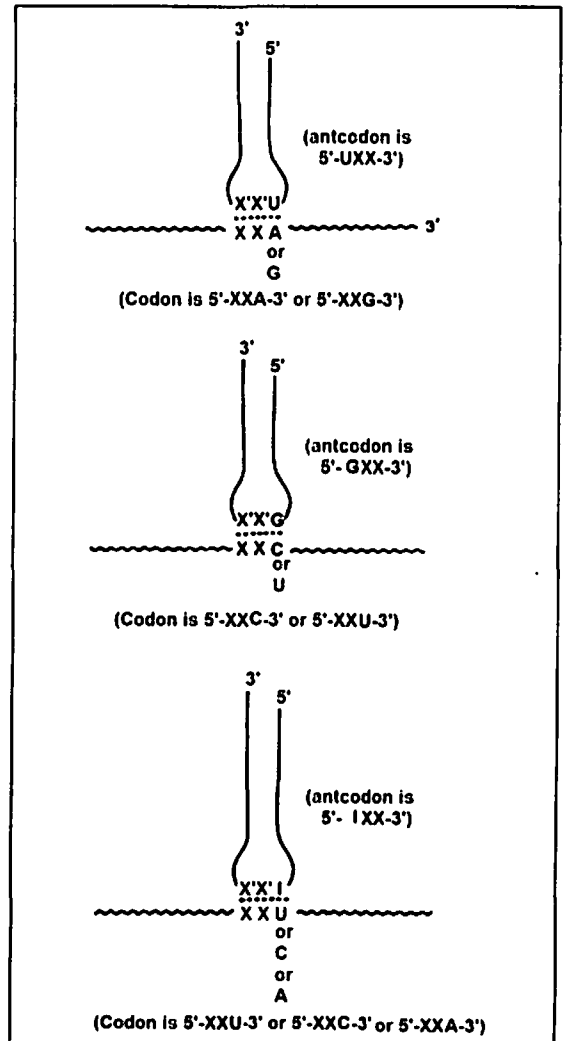


Figure 8.38: Wobble: Nontraditional base pairing between the 5'-nucleotide of the anticodon with the 3'-nucleotide of the codon.

c) This phenomenon called "wobble" and it allows a single tRNA to recognize several codons. The third base in the codon usually does not make any difference in the codon translation.

d) The result of this wobbling is that there is no need to 61 tRNA species in order to read the 61 codons coding for amino acids.

III. *Mutations:*

A. Definition

Mutation is the change in the sequence of nucleotide bases of the genetic code.

B. Causes of mutations:

1. Faulty replication of DNA.
2. **Physical agents** e.g. X-ray, and electromagnetic waves.
3. **Chemical agents** e.g. Free radicals and some anti-malignant drugs.
4. **Ionizing radiation.**

C. Types of mutations: There are two main types; point mutations and frame shift mutations.

1. Point mutations:

These are a **single base changes** where there is substitution (replacement) of one base for another.

a) **Types of point mutation:** It may be transitions or transversion:

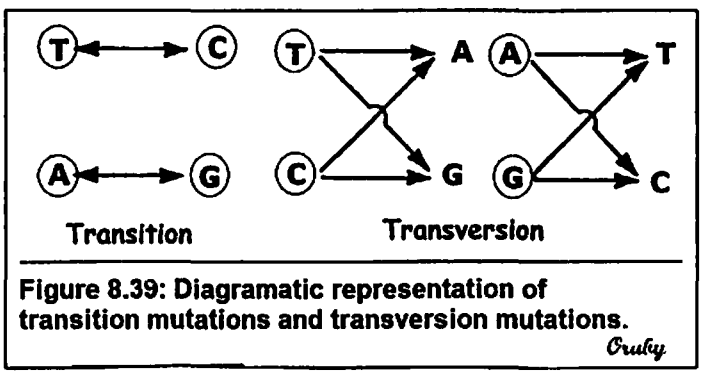


Figure 8.39: Diagramatic representation of transition mutations and transversion mutations.

Oraby

1) **Transition mutations:** One pyrimidine is changed to the other pyrimidine or one purine is changed to the other purine.

2) **Transversion mutation:** One purine is changed to either of the 2 pyrimidines or one pyrimidine is changed to either of the 2 purines (figure 8.39).

b) **Effects of point mutations:** It may lead to one of the following forms:

1) **Silent mutation:** The codon containing the changed base may still code for the **same amino acid.** For example if serine codon UCA is given a different third base (say, UCU), it will still code for serine. Therefore this is silent mutation.

2) **Missense mutation:** The codon containing the changed base may code for a **different amino acid.** For example , if the serine codon UCA is given a different first base (say, CCA), the codon will code for a different amino acid, in this case, proline. This type of mutation is called missense mutation. **The effect of the mistaken amino acid on the function of the protein molecule** depends upon the location of the substituted amino acid in that protein. It may be:

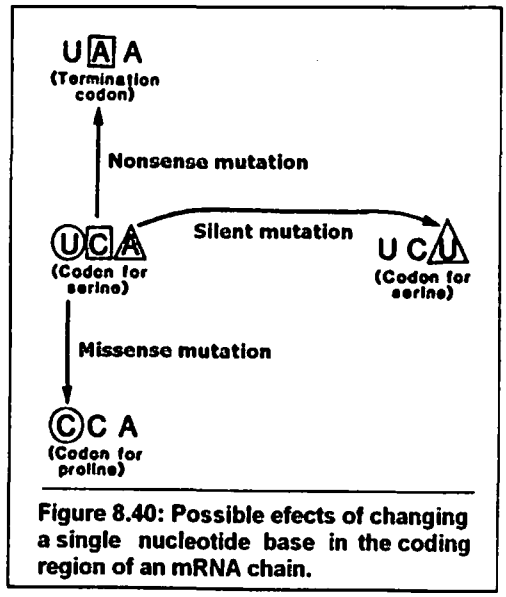


Figure 8.40: Possible efects of changing a single nucleotide base in the coding region of an mRNA chain.

i- **Acceptable;** producing functioning protein,

ii- **Partially acceptable**: producing less functioning protein.

iii- **Unacceptable:** producing non-functioning protein.

3) **Nonsense mutation:** The codon containing the changed base may become a **termination codon**, For example, if the serine codon UCA is given a different second base (say, UAA), the new codon will cause termination of translation at that point. The presence of termination codon at an inappropriate place is called: nonsense mutation.

c) **Example of point mutation:** Sickle cell anemia is a good example of point, transverse, missense, and partially accepted mutation:

1) Sickle cell anemia is a hemolytic disease in which red cells contain abnormal hemoglobin called: **hemoglobin S** (HbS).
2) HbS contains two normal alpha chains and two mutant beta chains in which **glutamate** at position **six** has been replaced by **valine** (figure 8.41).
3) This replacement results from the substitution of the base thymine **(T)** with adenine **(A)** in the 6th codon in gene of the beta globin *(point and transverse mutation)*. The 6th codon **(CTC)**, which codes for glutamate is changed into **(CAC)** which codes for valine *(missense mutation)*.
4) Glutamate is polar, while valine is non-polar amino acid and this single amino acid change leads to the formation of **HbS** *(less functioning, partially acceptable protein)* and makes RBCs fragile and easy to hemolyse.

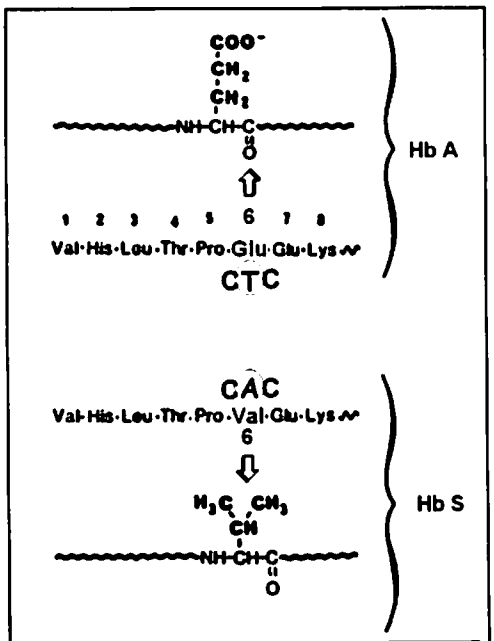


Figure 8.41: Substitution of valine for glutamate in hemoglobin S.

2. Frame shift mutations:

a) This type of mutation results from either **deletion or addition** of one or more nucleotide(s) in DNA that generates **altered mRNA.**

b) **Effects of frame shift mutation:** This may lead to:

1) **Garbled translation** of the mRNA distal to a single nucleotide deletion. (figure 8.36).
2) **Premature termination of polypeptide** due to appearance of nonsense codon near the -COOH terminus.
3) **Addition or deletion of amino acid to the protein:** if 3 nucleotides were inserted or deleted, this results in a protein in which one amino acid was missed or added.

IV. *Protein synthesis:*

A. Types and site of protein synthesis:

1. Body proteins are of various types e.g. plasma proteins, antibodies, contractile proteins of muscles, all enzymes and some hormones.
2. Protein synthesis in the body is subdivided among the various tissues e.g. mammary gland makes milk proteins, muscles make contractile proteins. etc.

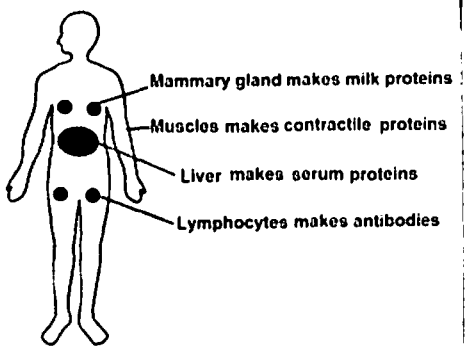


Figure 8.42: Types and site of protein synthesis.

B. Requirements for protein synthesis:

1. *Amino acids*
2. *tRNA*
3. *mRNA*
4. *Ribosomes*
5. *Aminoacyl-tRNA synthetase enzyme*
6. *Protein factors*

1. **Amino acids:** All amino acids that enter in the structure of protein must be present at the time of protein synthesis.
2. **Transfer RNA (tRNA):**
   a) At least one type of transfer RNA is required for each amino acid.
   b) **Some amino acids have more than one specific tRNA molecule. This is particularly true for those amino acids that have several codons.**
   c) **Each tRNA molecule contains a three-base nucleotides sequence called: anticodon that can recognize a specific codon on mRNA. This codon determines the type and the position of amino acid that will enter in the structure of protein molecule.**
   d) **Each tRNA molecule has an attachment site for specific amino acid at its 3` end. When a tRNA has a covalently attached amino acid, it is said to be charged. When tRNA is not bound to an amino acid it is defined as uncharged. The amino acid that is attached to the tRNA molecule is said to be activated.**
   e) **Because of their ability both to carry a specific amino acid and to recognize the codon for that amino acid, tRNA is known as adaptor molecule.**
3. **Messenger RNA (mRNA):** The specific mRNA required, as a template for the synthesis of the desired particular protein must be present.
4. **Ribosomes:**
   a) Ribosomes are large complexes of protein and rRNAs. They serve as factories responsible for protein synthesis.

b) The subunits of eukaryotic ribosomes (60S and 40S) and the containing rRNAs were discussed before in ***transcription of RNA***.

c) The complete ribosome has two binding sites for tRNA molecules P site and A site, each of which extends over both subunits. Together they cover two neighboring codons.

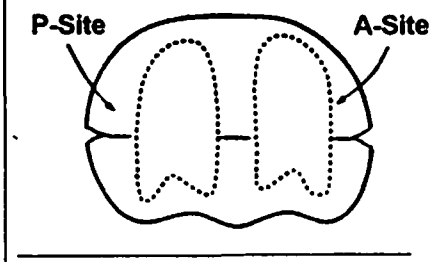


Figure 8.43: The complete ribosomes with its two binding sites.

5. Aminoacyl-tRNA synthetase enzymes:

a) Each member of this family of enzymes recognizes both a specific amino acid and the tRNA that corresponds to that amino acid (figure 8.44).

b) At least one member of this enzyme family is required for the **activation** of each type of amino acids.

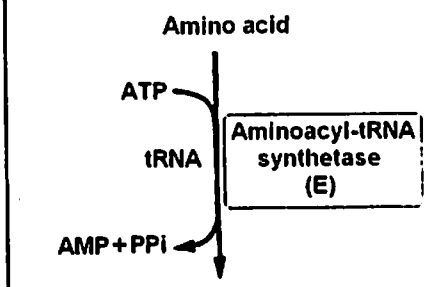


Figure 8.44: Attachment of a specific amino acid to its corresponding tRNA by aminoacyl-tRNA synthetase.

6. Protein factors:

a) Several proteins including initiation, elongation and termination factors are required for peptide synthesis.

7. High energy compounds: (ATP and GTP):

a) They are required as a source of energy.

V. *Steps of protein synthesis*: They can be divided into initiation, elongation and termination.

A. Initiation: (figure 8.45):

1. Initiation of protein synthesis begins by binding of mRNA to the ribosome,
2. **Requirements of initiation process:**
   - *a)* tRNA.
   - *b)* Ribosome.
   - *c)* mRNA.
   - *d)* Amino acids.
   - *e)* GTP and ATP.
   - *f)* At least 10 eukaryotic initiation factors (eIFs).
3. **Initiation can be divided into 4 stages:**
   - *a)* **Ribosomal dissociation:** Dissociation of the complete ribosome (80S) into its 40S and 60S:
     1) This step is carried out by two initiation factors (eIF-3 and eIF1A), which bind to 40S subunit, dissociating it from 80S and prevent its re-association.
   - *b)* **Formation of the 43S pre-initiation complex:** This complex is formed by binding of a ternary complex consisting of met-tRNA, GTP and eIF-2 to the 40S ribosome:
     1) The first step in this process involves the binding of GTP with eIF-2. This binary complex then binds to met-tRNA; to form a ternary complex. (Note: met-tRNA is specifically involved in binding to the initiation codon AUG).
     2) The ternary complex then binds to the 40S ribosomal subunit to form 43S pre-initiation complex.
   - *c)* **Formation of the 48S initiation complex:** This complex is formed by binding of mRNA to the 43S preinitiation complex:
     1) Initiation factor 4 subunits (eIF4) interact with mRNA cap.
     2) mRNA cap-together with eIF4-facilitate the binding of mRNA to the 43S pre-initiation complex.
     3) ATP hydrolysis occurs during mRNA binding.
     4) **Recognition of initiation codon:** Initiation occurs at the first **AUG** codon at 5` un-translated region of mRNA. The scanning mechanism starts by binding of

43S pre-initiation complex with mRNA near its 5` end. Then moves in the 3` direction until it meets the first AUG codon. ATP hydrolysis is essential for this scanning process.

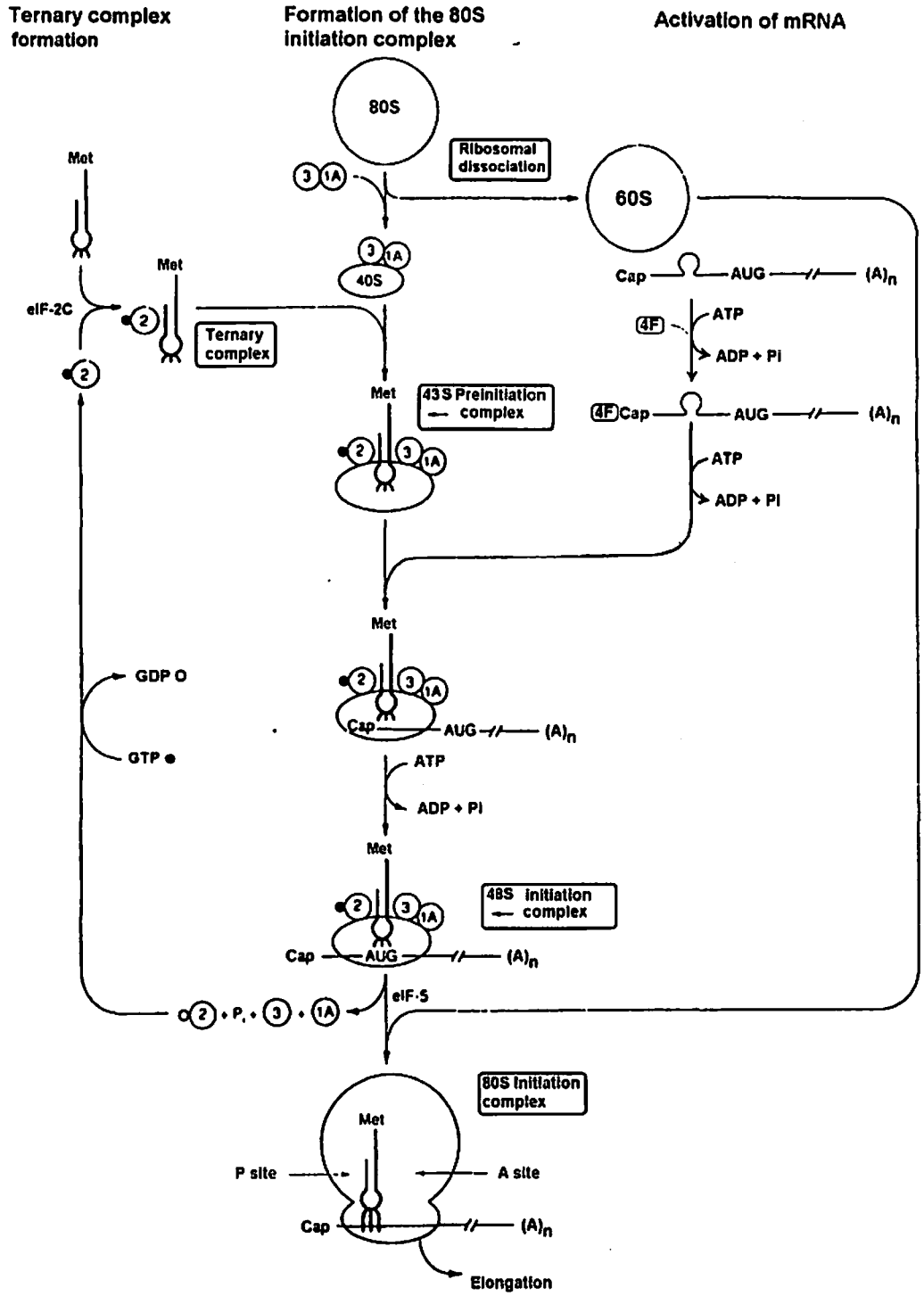


Figure 8.45: Diagramatic representation of the initiation of protein synthesis on the mRNA template containing a 5' cap and 3' poly (A). GTP=• and GDP=∘. The various initiation factors appear in abbreviated form as circles or squares e.g. eIF-3 = ③ and eIF-4F = 4F.

d) **Formation of the 80S initiation complex:** This complex is formed by binding of the 48S initiation complex with 60S ribosomal subunit:

1) This binding involves the hydrolysis of the GTP bound to eIF-2 by eIF-5, with release of initiation factors 1A, 2 and 3. These factors are then recycled.
2) The complete ribosome (80S) contains two sites for tRNA molecules. These are **P (peptide) site,** which is occupied by met-tRNA and **A site,** which is free and ready to receive the subsequent aminoacyl-tRNA.

B. Elongation:

1. Elongation of the polypeptide chain involves the addition of amino acids to the carboxyl end of the growing polypeptide chain.
2. During elongation the ribosome moves from the 5`end to the 3 end of the mRNA.
3. **Requirements for elongation include:**
   a) 80S initiation complex.
   b) Aminoacyl-tRNAs.
   c) GTP.
   d) Eukaryotic elongation factors , eEF-1α and eEF-2.
   e) met-tRNA is present in P site (from initiation).
4. **Stages of Elongation can be divided into 3 stages:**
   a) **binding of aminoacyl-tRNA to the A site:**
      1) Entry of a new aminoacyl-tRNA to the empty A site on the ribosome requires proper codon recognition. This depends on the complementary bases of both codon and anticodon.
      2) At first eEF-1α forms a complex with GTP and the entering aminoacyl-tRNA.
      3) This complex allows the aminoacyl-tRNA to enter the A site with the release of eEF-1α and hydrolysis GTP into GDP and phosphate. These factors are then recycled.
   b) **Peptide bond formation:**
      1) The carboxyl group (-COOH) of the aminoacyl-tRNA in P site is transferred to and bind with the amino (NH_2) group of the new aminoacyl-tRNA in the A site. This reaction is catalyzed by peptidyl transferase enzyme present in 60S subunit.

c) Translocation:

1) After the peptide bond has been formed, the ribosome moves three nucleotides toward the 3`end of mRNA.
2) This process is known as translocation and it requires translocase enzyme, eEF-2 and GTP.
3) As a result of movement of ribosomes, the following events occur:
   - i- Release of the uncharged tRNA from P site of ribosome.
   - ii- Transfer of the newly formed peptidyl tRNA from A site to occupy P site.
   - iii- A site becomes free. Thus it can be occupied by another new aminoacyl tRNA according to the codon-anticodon recognition. Then the process will be repeated.

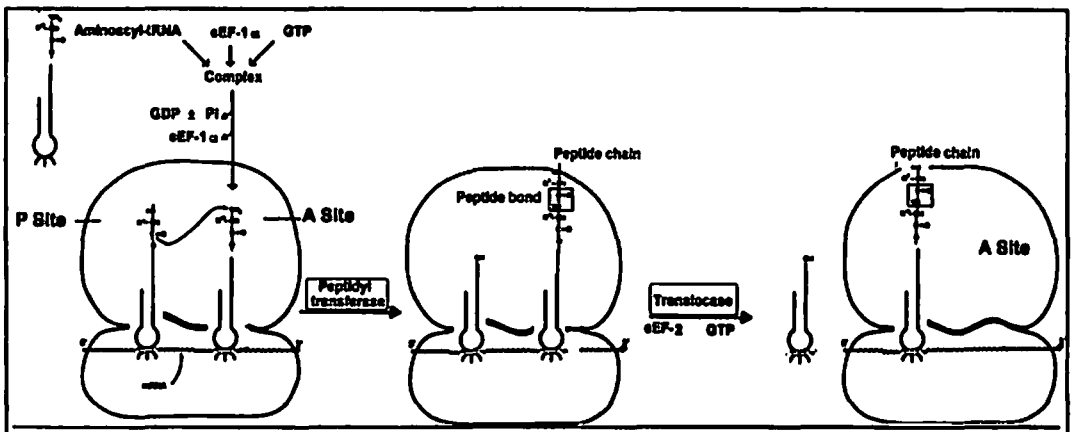


Figure 8.46: Binding of aminoacyl-tRNA to the A site and peptide bond formation, followed by movement of ribosomes towards 3' end of mRNA by translocase enzyme. Oraby

4) **For each new peptide bond formed, 4 high energy phosphate bonds are cleaved:**
   - i- The charging of the tRNA molecule with the aminoacyl group requires the hydrolysis of an ATP to an AMP + PPi. This equivalent to the hydrolysis of **2ATPs** to 2ADP + 2Pi.
   - ii- The entry of the aminoacyl-tRNA into the A site leads to the hydrolysis of one **GTP** to GDP + Pi.

iii- The translocation of the newly formed peptidyl tRNA in the A site into the P site by eEF-2 similarly leads to the hydrolysis of one **GTP** to GDP + Pi.

5) First initiator methionine amino residue, coded by **AUG** is removed at the N-terminal end before synthesis of the polypeptide chain is completed, unless it is the required N-terminal amino acid for the polypeptide.

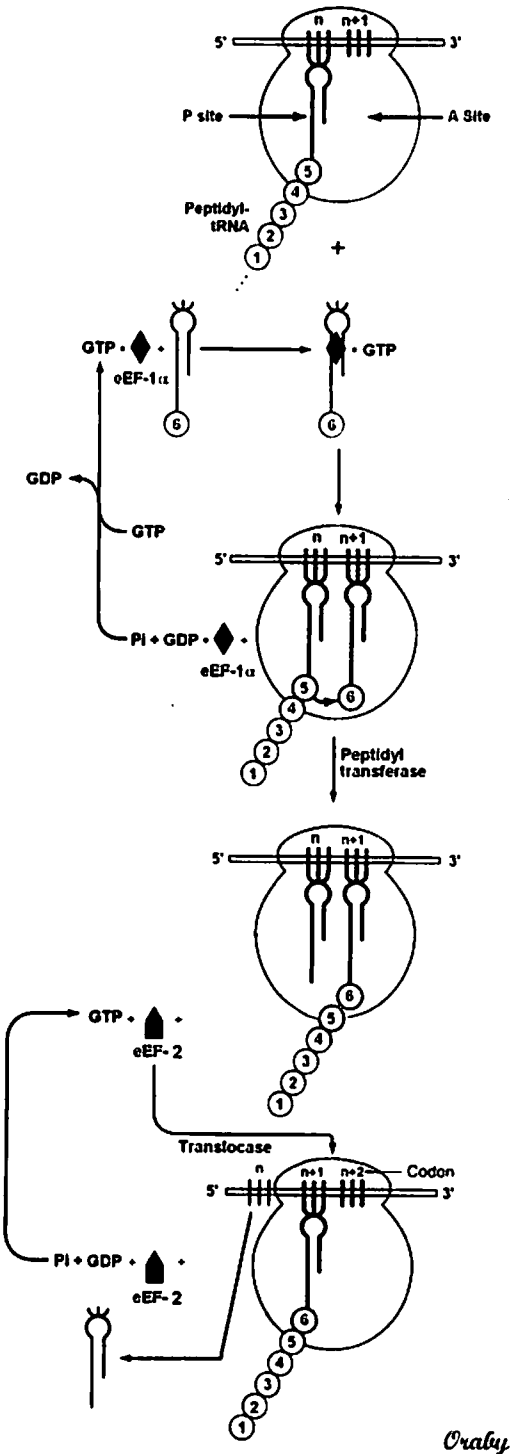


Figure 8.47: Diagrammatic representation of the peptide elongation process of protein synthesis.

The small circles labeled n – 1, n, n + 1, etc. represent the amino acid residues of the newly formed protein molecule. eEF-1α and eEF-2 represent elongation factors 1 and 2, respectively. The peptidyl-tRNA and aminoacyl-tRNA sites on the ribosome are represented by P site and A site, respectively.

C. Termination:

1. Termination is the final step, which occurs after multiple cycles of elongation and formation of protein molecules.
2. Termination occurs when ribosome moves to bring one of the three **termination (nonsense) codons** into A site. These codons are **UAA, UAG and UGA.**
3. **Releasing factors (eRF) 1,2 and 3** which are present in A site can recognize all the three termination codons.
4. Releasing factors together with GTP and peptidyl transferase promote the hydrolysis of the bond between peptide and tRNA occupying the P site .**This hydrolysis leads to :**
   a) Release of both peptide and tRNA.
   b) Dissociation of 80S ribosomes into its 40S and 60S subunits, which are then recycled.

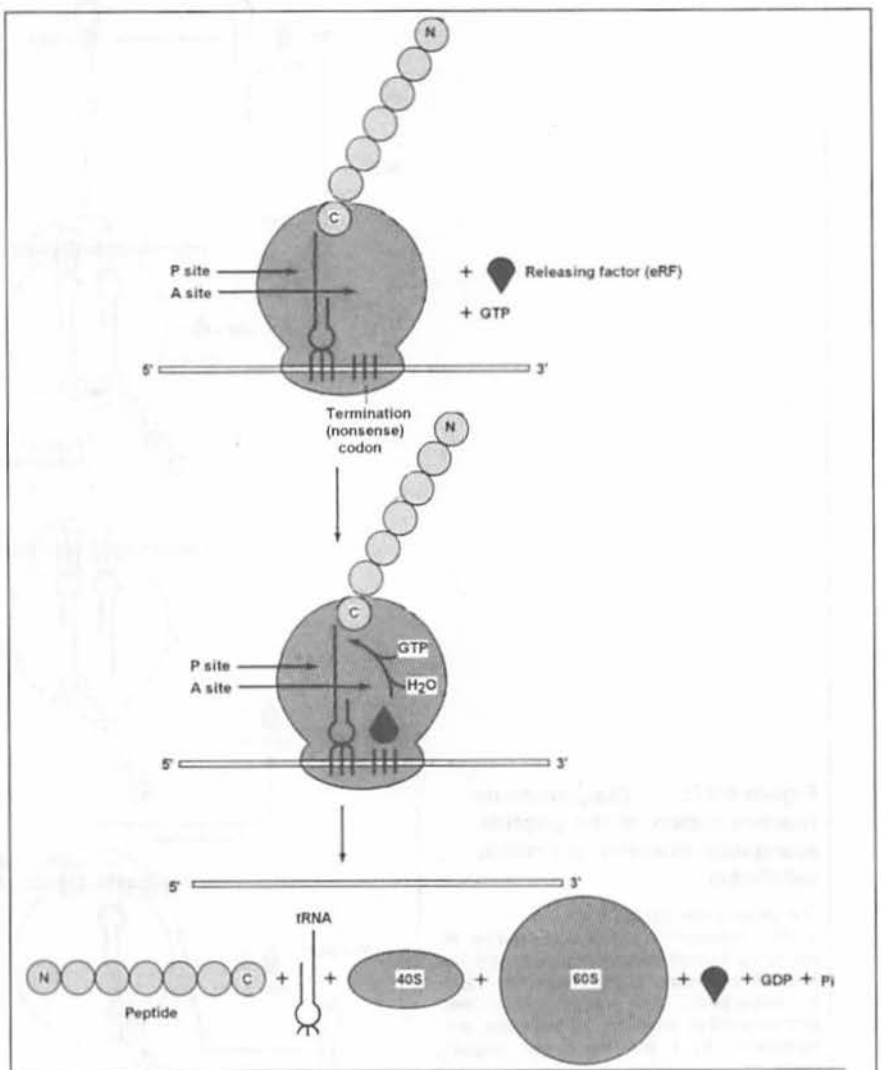


Figure 8.48: Diagrammatic representation of the termination process of protein synthesis.
The peptidyl-tRNA and aminoacyl-tRNA sites are indicated as P site and A site, respectively. The hydrolysis of the peptidyl-tRNA complex is shown by the entry of H_2O. N and C indicate the amino and carboxyl terminal amino acids, respectively, and illustrate the polarity of protein synthesis.

VI. *Polyribosomes (polysomes):* Because the length of the nucleotide sequence of most mRNAs, more than one ribosome can translate the same mRNA at the same time. Such a complex of one mRNA and a number of ribosomes is called a polyribosome or **polysome.**

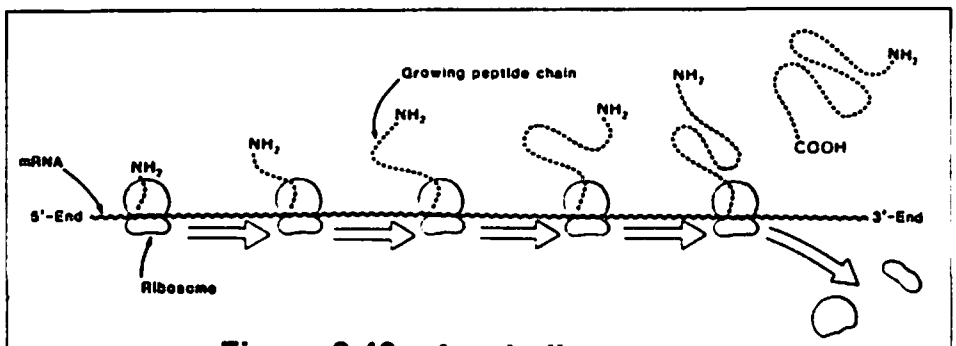


Figure 8.49: A polyribosome

Summary of events of protein synthesis:

I. *Activation of amino acids* :

A. This is accomplished by a family of enzymes called **aminoacyl-tRNA synthetase.**

B. Each aminoacyl-tRNA synthetase enzyme catalyzes a two steps reaction that results in attachment of the amino acid to the 3` terminus of tRNA. The overall reaction requires ATP, which is cleaved to AMP + PPi.

II. *Initiation:* It can be divided into 4 stages:

A. Ribosomal dissociation :

Complete ribosome (80S) $\xrightarrow{\text{eIF-3 + eIF-1A}}$ 40S + 60S

B. Formation of 43S preinitiation complex:

Ternary complex (GTP + eIF-2 + met-tRNA) + 40S ➔ Preinitiation complex

C. Formation of the 48S initiation complex:

eIF-4F + mRNA + 43S preinitiation complex $\xrightarrow{\text{ATP}}$ 48S intiation complex

D. Formation of the 80S initiation complex:

48S Initiation complex + 60S $\xrightarrow[\text{eIF-2 eIF-3 GDP eIF-1A}]{\text{EIF-5}}$ 80S with met-tRNA at P site

III. *Elongation:* It can be divided into 3 stages:

A. Binding of aminoacyl-tRNA to the A site:

eEF-1 + GTP + proper aminoacyl-tRNA (according to codon - anticodon recognition). This complex allows the aminoacyl-tRNA to enter the A site with release of eEF-1 and hydrolysis of GTP into GDP and Pi.

B. Peptide bond formation:

The -COOH group of the aminoacyl-tRNA in P site is transferred to and bind with the $-NH_2$ group of the new aminoacyl-tRNA in the A site.This reaction is catalyzed by peptidyl transferase enzyme in 60S subunit.

C. Translocation:

1. After the peptide bond has been formed, the ribosome moves three nucleotides toward the 3` end of mRNA. This process needs translocase enzyme, eEF-2 and GTP.
2. As a result of movement of ribosome, the following events occur:
   a) Release of uncharged tRNA from P site of ribosome.
   b) Transfer of the newly formed peptidyl-tRNA from A site to occupy P site.

c) A site becomes free. It can be occupied by another new aminoacyl-tRNA according to codon and anticodon recognition. The process will be then repeated.

3. Four high energy bonds (2 from ATP and 2 from GTP) are hydrolyzed) for each peptide bond formed.

IV. ***Termination:***

A. Termination occurs when ribosome move to bring one of the three termination codons into the A site. These codons are UAA, UAG and UGA.

B. Releasing factors (eRF) 1, 2 and 3 (present in A site) can recognize the three termination codons.

C. eRF , GTP and peptidyl transferase promote the hydrolysis of bond between the peptide and tRNA occupying the P site . This hydrolysis leads to:

1. Release of both peptide and tRNA.
2. Dissociation of 80S ribosome into its 40S and 60S subunits which are then recycled .

VII. ***Posttranslational changes:*** These are modifications (changes), which occur to protein after translation. Examples of these modifications include:

A. **Conversion of inactive protein to active one (protein trimmings):** The protein precursor may undergo a wide variety of proteolytic cleavages (N- and C-terminal trimmings). Some enzymes are synthesized as inactive proteins (zymogens). They are converted into active enzymes by removal of a polypeptide chain e.g. the inactive pancreatic trypsinogen (zymogen) becomes active trypsin (enzyme) in the small intestine. Insulin hormone is also synthesized as inactive proinsulin.

B. **Glycosylation i.e. addition of carbohydrate:** Many proteins e.g. cell membrane proteins receive carbohydrate chains, which are attached to serine or threonine (-OH) group or to asparagine (N) group. This process occurs in Golgi apparatus and results in formation of **glycoprotein.**

C. **Hydroxylation i.e. addition of -OH group:** e.g. proline and lysine amino acids of collagen are hydroxylated into hydroxy proline and hydroxy lysine. Vitamin C is essential for this process.

D. **Phosphorylation i.e. addition of phosphate:** It occurs on the (-OH) group of serine or threonine residues in protein. Phosphorylation may lead to either activation or inactivation of some proteins e.g. glycogen synthase enzyme is inactivated by phosphorylation, whereas glycogen phosphorylase enzyme is activated by this process (see carbohydrate metabolism).

VIII. *Inhibition of protein synthesis in prokaryotes:*

(by antibiotics and diphtheria toxins):

A. **Antibiotics:** Ribosomes in bacteria are smaller than that of eukaryotes (70S rather than 80S). Bacteria also have simpler components of RNA and protein molecules. These allow many antibiotics to **react specifically** with bacterial ribosomes and thus inhibit protein synthesis. This results in bacterial death without harmful effect on eukaryotic cells. **Examples of these antibiotics are:**

1. **Streptomycin:** It binds to the ribosome, distorting its structure. It causes dissociation of mRNA from the ribosomes.
2. **Tetracyclines:** They interact with small ribosomal subunits and prevent aminoacyl-tRNA anticodons from recognizing their corresponding codons.
3. **Chloramphinicol:** It inhibits peptidyl transferase enzyme.
4. **Puromycin:** Its structure resembles the structure of aminoacyl-tRNA. It becomes incorporated into the growing peptide chain, thus causing inhibition of further elongation.

B. **Diphtheria toxins:** It is an exotoxin produced by a bacterium called **corynbacterium diphtheria.** These toxins inactivate the eukaryotic elongation factor-2 (eEF-2), thus preventing translation.

Regulation of gene expression

I. Every organism has the capacity to synthesize a large number of different proteins, and because those proteins are needed in different amounts and at different times, **gene expression** must be regulated. For example, the bacterium Escherichia coli (E.coli) contains genes for about 3000 different proteins, but it does not need to synthesize all of these proteins at the same time or in the same amount.

II. Thus prokaryotic and eukaryotes cells have a mechanisms for regulation of protein synthesis.

Regulation of Prokaryotic Genes (Lactose 'lac' operon)

I. *Introduction:*

A. E. Coli bacteria can regulate synthesis of enzymes that utilize lactose at transcriptional level. This can be achieved by a set of 3 genes, which are structural genes, control genes and regulatory gene (fig. 8.50).

B. The lactose (lac) operon will be taken as an example for regulation of protein biosynthesis in prokaryotes. Two scientists named **Jacob**

and **Monod** in 1961 explained this model of regulation.

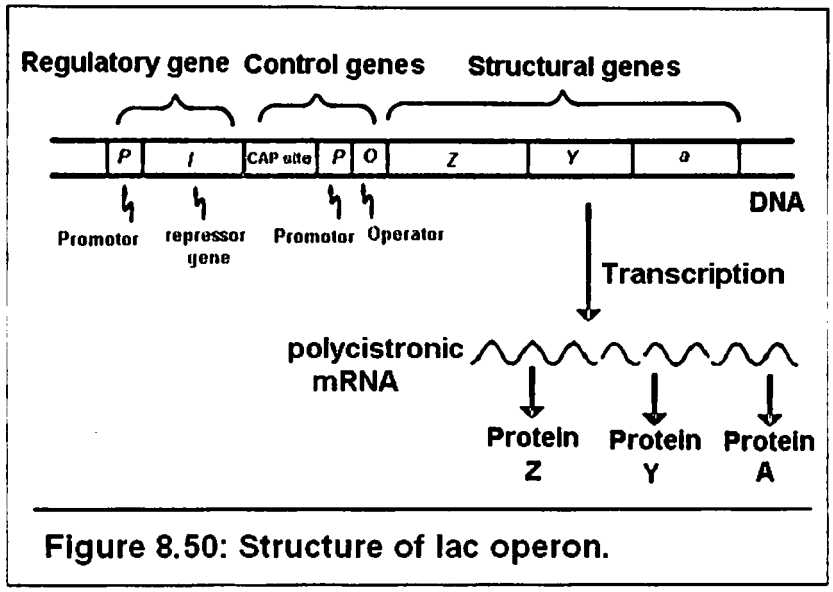


Figure 8.50: Structure of lac operon.

II. *Relationship of protein synthesis to nutrient supply:*

A. Prokaryotes e.g E. coli require a source of energy, which is usually obtained from oxidation of a sugar (glucose, lactose, and galactose).

B. However, E.coli prefer the use of **glucose** than other sugars because its metabolism requires fewer enzymes.

C. In absence of glucose, E. coli use lactose as a source of energy. (*Therefore and for economy and saving energy, E.coli bacterium synthesizes enzymes required for catabolism of lactose* ***only*** *when lactose is available and glucose is absent).*

D. In order to metabolize lactose, E.coli must synthesize 3 enzymes:

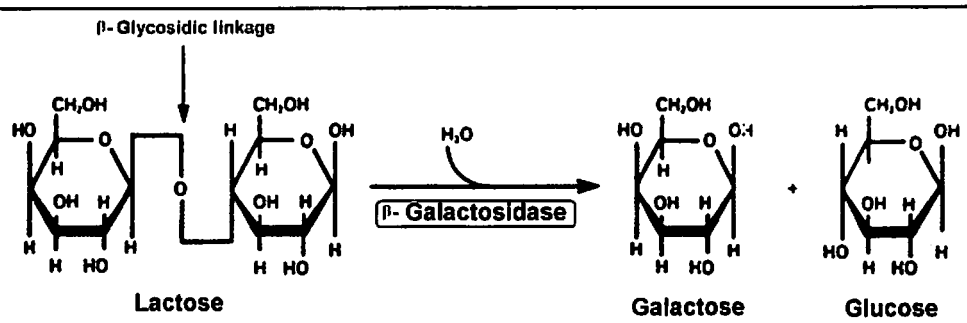


Figure 8.51: The hydrolysis of lactose to galactose and glucose by the enzyme β- galactosidase.

(**β-galactosidase**; which hydrolyzes lactose into glucose and galactose (figure 8.51), **lactose permease**; which transport lactose into the cell and **galactose acetylase** whose function is not understood.

III. *Lactose (lac) operon includes:*

A. **Structural genes,**

1. These are a **group of 3 genes** (z, y and a) adjacent to each other in the genome. They are **coordinately controlled**; that is, the genes are either all transcribed or all not transcribed.
2. The structural genes of lac operon (z, y and a) are transcribed from a single promotor **and translated into** a series of different **protein enzymes.**
3. The resulting mRNA contains 3 units of translation (cistrons) and is referred to as a polycistronic mRNA. This mRNA is translated into 3 different proteins:
   a) β-Galactosidase.
   b) Lactose permease.
   c) Galactose acetylase.

B. **Control genes:**

Are sites on DNA near the structural genes. They include three regions: operator (O), promoter (P) and CAP regions. They control the transcription of structural genes.

1. **Operator region (O)**, to which lac repressor binds.
2. **Promotor region (P)**, which is the site where RNA polymerase is bound.
3. **Catabolite activator protein (CAP) region:** to which CAP and cAMP bind.

C. **Regulatory repressor gene (i): Which is transcribed and translated into a protein called the lac repressor.**

IV. *The transcription of Lac structural genes into mRNA needs the following conditions:*

A. Absence of glucose

B. Operator: should be free of repressor.

C. CAP site: should bind with cAMP-CAP complex.

D. Promotor: should bind with RNA polymerase.

V. *Repression and repressors:*

A. **Repression** is the process whereby a protein called a repressor binds to operator site and inhibits transcription of operon.

B. Repressor:

1. It is a **protein** synthesized under control of regulatory repressor gene (i).
2. This protein is continuously produced because lac repressor gene is a **constitutive** gene i.e. gene which maintains a constant rate of protein synthesis.
3. Binding of a repressor protein to the operator region (O) prevents binding of RNA polymerase to the promoter (P) and inhibits transcription of the structural genes of operon, figure 8.52.
4. The repressor thus shows **negative control** on expression of the operon.

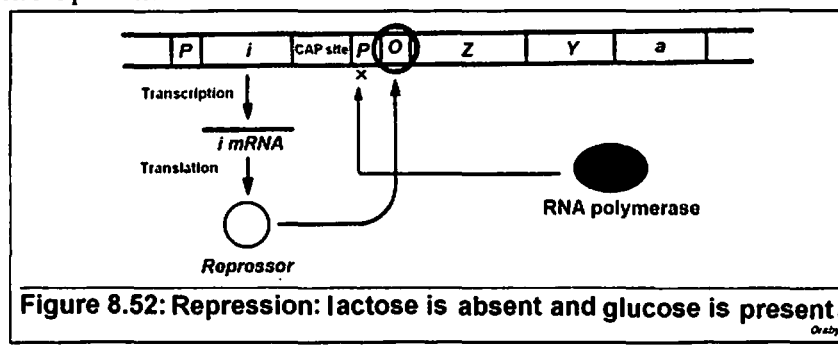


Figure 8.52: Repression: lactose is absent and glucose is present.

VI. *Induction and inducers:*

A. **Induction** is the process whereby an inducer stimulates transcription of an operon.

B. **Inducers:**

1. They are usually substrates (sugars) for enzymes that will be transcribed and translated by structural genes e.g. **lactose** is the inducer for lac operon
   a) They may also be compounds **similar to** the substrates e.g allolactose, which is a metabolite of lactose. These compounds are called **gratuitous inducers.**
   b) The inducer binds to the repressor, inactivating it.
   c) The **inactive repressor does not bind** to operator.
2. **RNA polymerase** therefore, can bind to the promotor and transcribe the operon → protein enzymes (β-galactosidase, permease and transacetylase).
3. The lac operon is induced only in absence of glucose even if lactose (inducer) is available (see later).

VII. *Catabolite activating protein (CAP) site:*

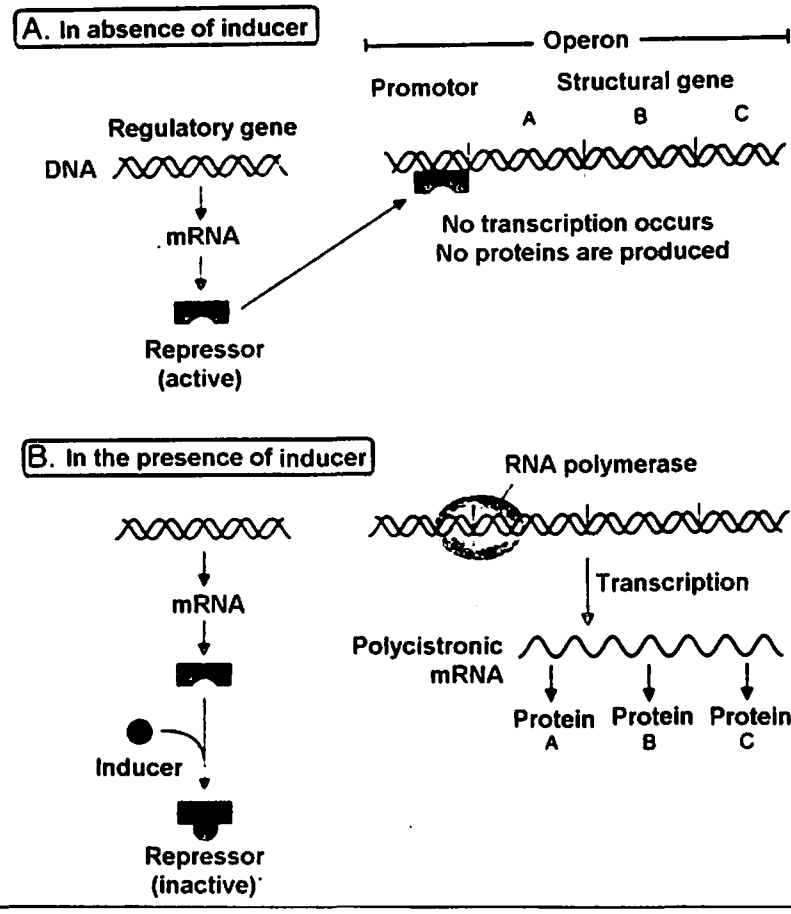


Figure 8.53: An inducible operon (e.g. lac operon) If the inducer is absent, the repressor is active and binds to the operator, preventing RNA polymerase from binding. Thus, transcription does not occur. If the inducer is present, it binds to and inactivates the repressor, which then does not bind to the operator.

A. CAP site is one of control genes of lac operon. For transcription to occur, CAP site should be occupied by cAMP-CAP protein complex. This facilitates binding of RNA polymerase to the operator site.

B. **<u>cAMP is increased in absence of glucose:</u>**

1. When glucose decreases, cAMP levels rise (**↓Glucose → ↑cAMP**).
2. When glucose is available, cAMP levels decreases (**↑Glucose → ↓cAMP**).
   a) cAMP binds to the catabolite activating proteins **(CAP) → cAMP-CAP complex.**
   b) The **cAMP-CAP** complex binds to the **CAP site** near the promotor of the operon **—►facilitates** binding of RNA polymerase **—►**Transcription of mRNA for enzymes that allow cells to utilize lactose.

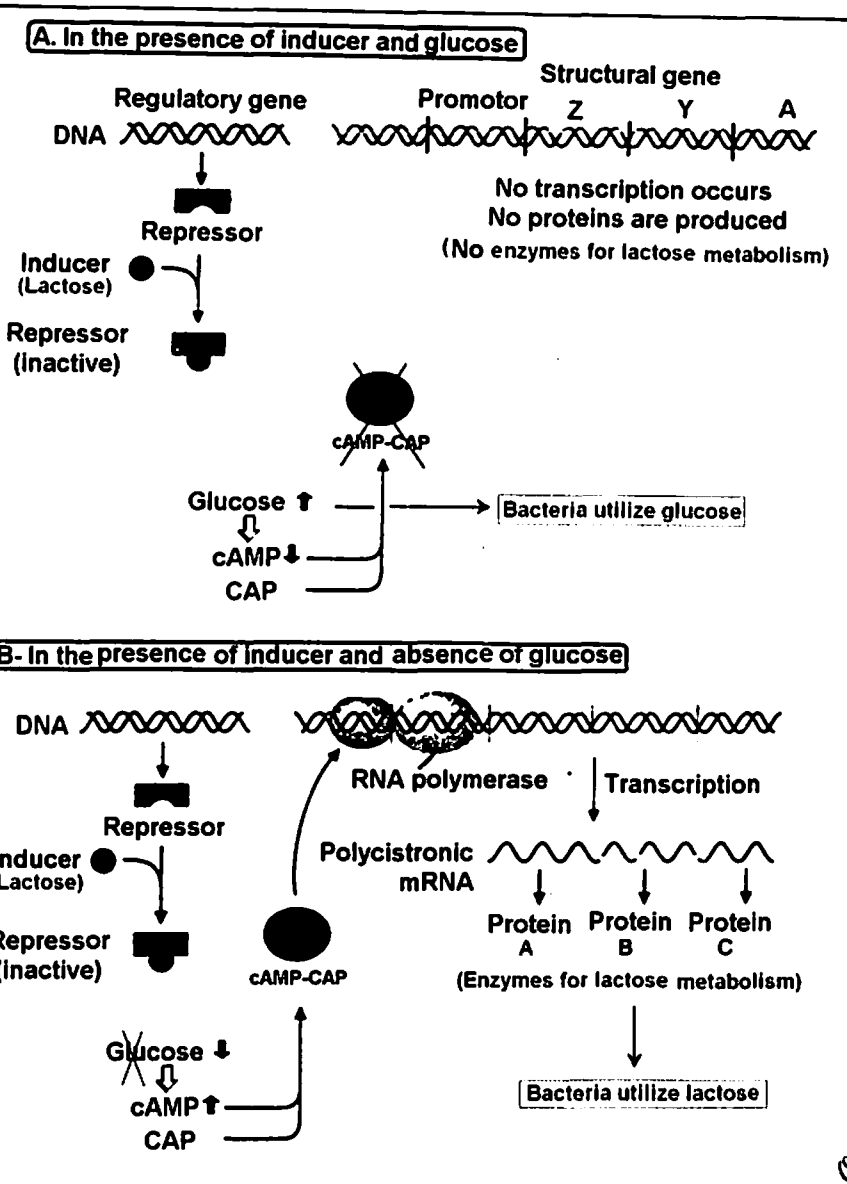


Figure 8.54: Transcription occurs in the presence lactose and absence of glucose.
The operon is transcribed only when glucose is low. Cyclic adenosine monophosphate (cAMP) is elevated, and the inducer binds to the repressor, inactivating it. Under these conditions, the cAMP-CAP complex forms and binds to the DNA, facilitating the initiation of transcription by RNA polymerase. The *lac* operon exhibits catabolite repression.

Conclusion

- *Cells of E. coli use glucose as a source of energy when it is available even if lactose is present.*
- *In the presence of glucose and lactose, the lac repressor is inactivated by lactose but the levels of cAMP is low → **No transcription.***
- *In the presence of lactose and absence of glucose:*
  - ***cAMP is high:** due to absence of glucose.*
  - ***The lac repressor is inactivated** due to presence of lactose as Inducer.*
- *These facilitate binding of RNA polymerase to the promotor*
- *Transcription of mRNA for enzymes that allow cells to utilize lactose.*

Regulation of Eukaryotes Genes

I. Introduction:

Eukaryotes also regulate gene expression through changes in genes or from mechanisms that affect transcription, modification and transport of mRNA or mRNA translation.

A. Changes in genes:

1. **Gene can be lost** (or partially lost) from cells, so that functional proteins cannot be produced e.g. during differentiation of red blood cells.
2. **Genes can be amplified:** for example, the drug methotrexate causes production of hundreds of copies of the gene for the enzyme dihydrofolate reductase. This leads to resistance to the drug (see folic acid, vitamins, part I).
3. **Segments of DNA can move from** one location to another on the genome, e.g. during differentiation of lymphocytes, specific genes are selected and rearranged so that they are adjacent to each other in the genome and can act as a single transcriptional unit for a specific antibody.

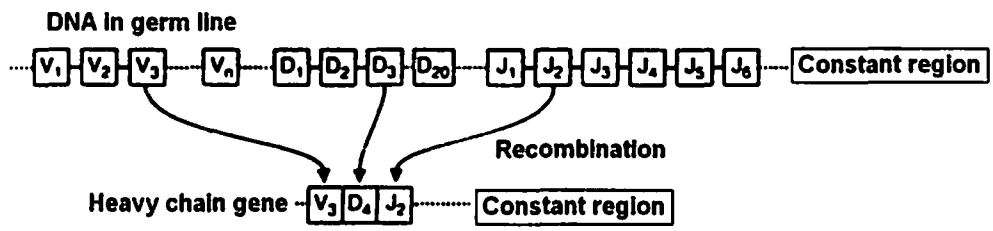


Figure 8.55: Rearrangement of DNA.
Specific V, D, and J segments from among a large number of potential sequences in the DNA of precursor cells combine to form the heavy chain gene from which lymphocytes produce immunoglobulins (antibodies).

4. **Methylation of bases in DNA** affects the transcriptional activity of a gene:
   a) Cytosine can be methylated at its 5 position. This methylation inhibits transcription.
   b) Globin genes are more methylated in non-red cells (nonerythroid) than in red cells (erythroid), in which they are expressed.

B. Regulation at the level of transcription:

1. **Enhancers:** e.g. steroid hormones enter cells, bind to protein receptors and **activate specific genes.**
2. **Histones,** which are small basic proteins associated with the DNA of eukaryotes may act as nonspecific repressor.
3. **Some genes have more than one promotor.** Thus the

promotor that is used can differ under different physiologic conditions or different cell types.

C. **Regulation by post-transcriptional modification:**

1. Regulatory mechanisms that occur during capping, poly A tailing and splicing can alter the amino acid sequence or the quantity of the protein produced from the mRNA.

D. **Regulation at the level of the translation**, during the initiation or elongation reactions:

1. **Heme** stimulates the synthesis of globin by inhibiting the phosphorylation of initiation factor 2 (eIF-2), causing stimulation of initiation.
2. **Insulin hormone** stimulates the phosphorylation of eIF-4, causing inhibition of initiation.

Molecular Biology Techniques

These are different techniques using amplified DNA molecules. All these techniques will be discussed.

I. *DNA amplification:*

A. Definition:

These are techniques used to give thousand or million copies of a particular gene. The aim is to study these ampilified genes.

B. Types of DNA amplifications:

1. **In vivo** i.e. inside living cells. This is known as recombinant DNA technology and molecular cloning.
2. **In vitro** i.e. inside the test tubes e.g. polymerase chain reaction (PCR).

In vivo techniques for DNA amplification Recombinant DNA technology (Genetic engineering):

I. *Introduction:*

A. Definitions:

1. **Recombinant DNA technology:** is the technique by which 2 different DNA strands (human and bacterial) are coupled together giving rise to a new DNA molecule called recombinant DNA (=chimeric DNA or hybrid DNA).

2. **Recombinant DNA:** is a new DNA molecule, which contains both human and bacterial DNA sequences i.e. contains genetic information from human and bacterial DNA.

B. **Example:** If insulin gene is taken and inserted into the DNA of E. coli bacterium:

1. The new DNA is a recombinant DNA.
2. The insulin gene is called **donor** and the bacterial DNA is called **vector.**
3. The bacterial cells divides very rapidly making billions of copies of bacteria, carrying the insulin gene i.e. E. coli cells has inherited the human insulin gene.

II. *Creation of recombinant DNA:*

A. Requirements for preparation of chimeric DNA:

1- Donor DNA
2- Cloning vectors:
a. Plasmids
b. Phages
c. Cosmids
3- Restriction enzymes
4- Ligase enzyme

1. **Donor:**
   This is the DNA segment of interest (donor DNA), which is chosen and inserted in a carrier DNA (vector).
2. **Cloning vector:** This is the carrier part of the recombinant DNA molecule. It must have two **important properties** for its functions: the ability to enter the cell and the ability to replicate. The vector is either bacterial plasmid, phage or cosmid:
   a) **Bacterial plasmids:** These are extra-chromosomal small circular, duplex DNA molecules.
      1) **The natural function of plasmids** is to give antibiotic resistance to the host cells.
      2) **Causes of using plasmids as a cloning vectors:**
         i- They replicate independently from the bacterial DNA and exist as single or multiple copies within the bacterium.
         ii- The complete DNA sequence of many plasmids is known; hence the exact location of restriction

enzyme cleavage sites for inserting the donor DNA is available.

iii- Plasmids are easily separated from the bacterial chromosomes because they are smaller than them.

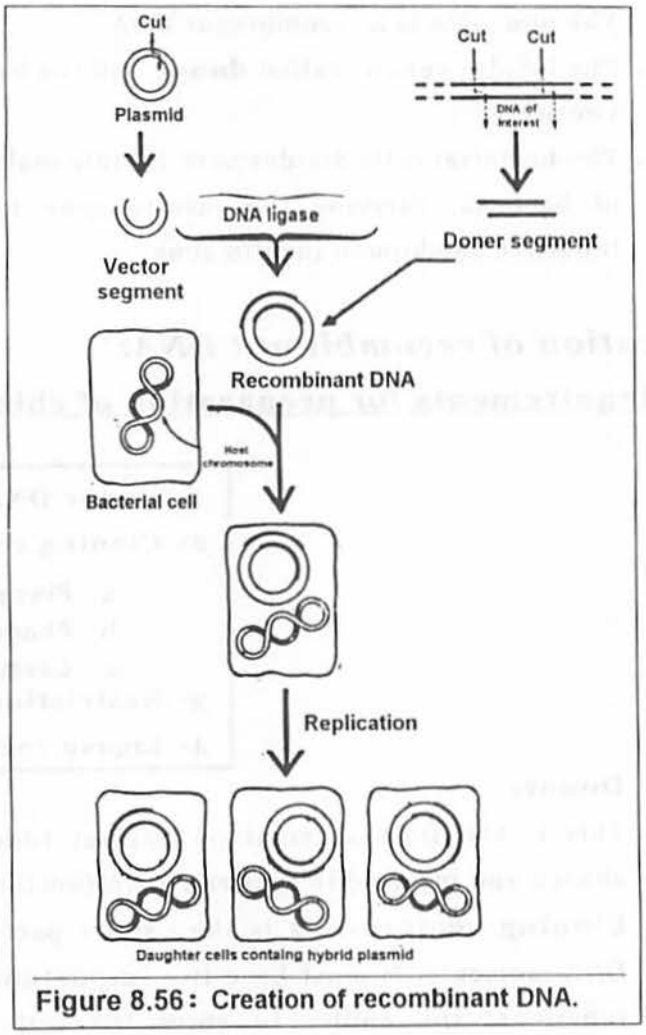
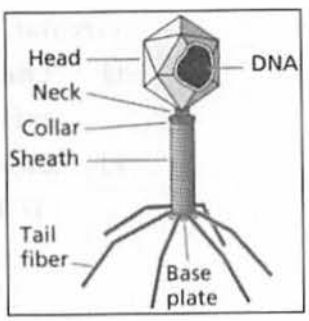


Figure 8.56: Creation of recombinant DNA.

b) **Phages:**

1) They are type of viruses that live in bacteria.
2) They usually have linear DNA molecules into which donor DNA can be inserted at several restriction enzyme sites.
3) Many phages contain one or more proteins in addition to nucleic acid.



c) **Cosmids:** These are plasmids that act as vectors for larger fragments of DNA than do both phages and other plasmids.

3. **Restriction endonucleases (or restriction enzymes):** These are a very important enzymes in the preparation of both the donor and vector segments of the recombinant DNA molecules:

a) Restriction endonucleases are **bacterial enzymes** that recognize and act on a short part of DNA (4 or 6 base pairs).

b) These short parts contain specific DNA sequences, which differ for each restriction endonuclease. They have **twofold rotational symmetry** i.e. palindrome (figure 8.57). This means that within a short region of the double helix , the nucleotide sequence on the "top" strand , read in the 5` to 3` is identical to that of the "bottom" strand also read in the 5` to 3`.

c) Restriction endonucleases make highly specific cuts in the previous short specific sequences of DNA, one in one strand and one in the other. These cuts are of 2 types:

1) **Blunt ends:** For example, the bacterial enzyme Hae II makes cuts as shown in figure 8.57, breaking the phosphate backbone of the two strands at the axis of symmetry.

2) **Staggered ends:** For example the bacterial enzyme EcoRI breaks the phosphate backbones in such a way that each strand has an overlapping part or **sticky ends.**

d) Restriction endonucleases do not degrade the DNA of bacterial cells because the sites recognized by them are methylated by special DNA methylase enzymes. Such methylation prevents DNA degradation.

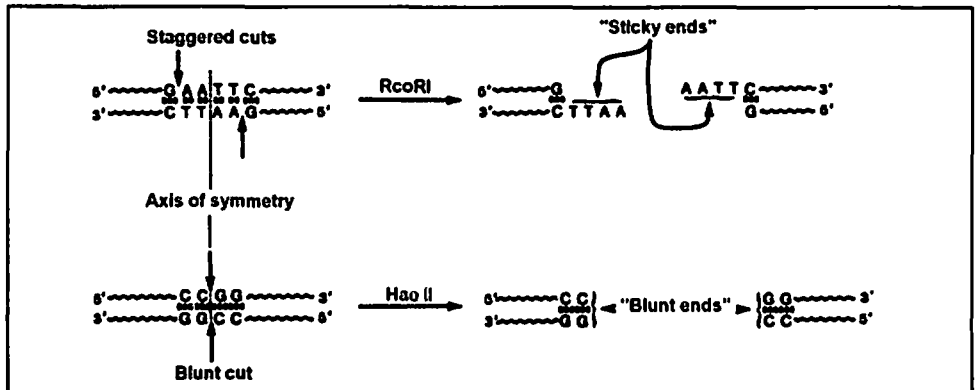


Figure 8.57: Examples of restriction endonucleases:
Examples of *restriction endonuclease* cleavage sites on DNA showing twofold, rotational symmetry. (Cleavage sites shown are those of the restriction endonucleases *Eco R1* and *Hae II*). The cleavage sites are found at the arrows. The axes of symmetry are marked by dotted lines.

B. Preparation of chimeric DNA molecules:

1. **Selection of donor DNA:** DNA segment of interest (donor DNA) is chosen and cleaved by restriction endonuclease.
2. **Selection of vector:** A cloning vector is chosen (plasmid, phage or cosmid).
3. **Integration of donor DNA with vector DNA** (e.g. plasmid): This can be done by one of the following procedures:

a) **Sticky ends:** are formed between the donor DNA and that of the vector (figure 8.58). The 2 ends anneal together by the base pairing rule. The ends are attached together by DNA ligase to form recombinant (hybrid) DNA.

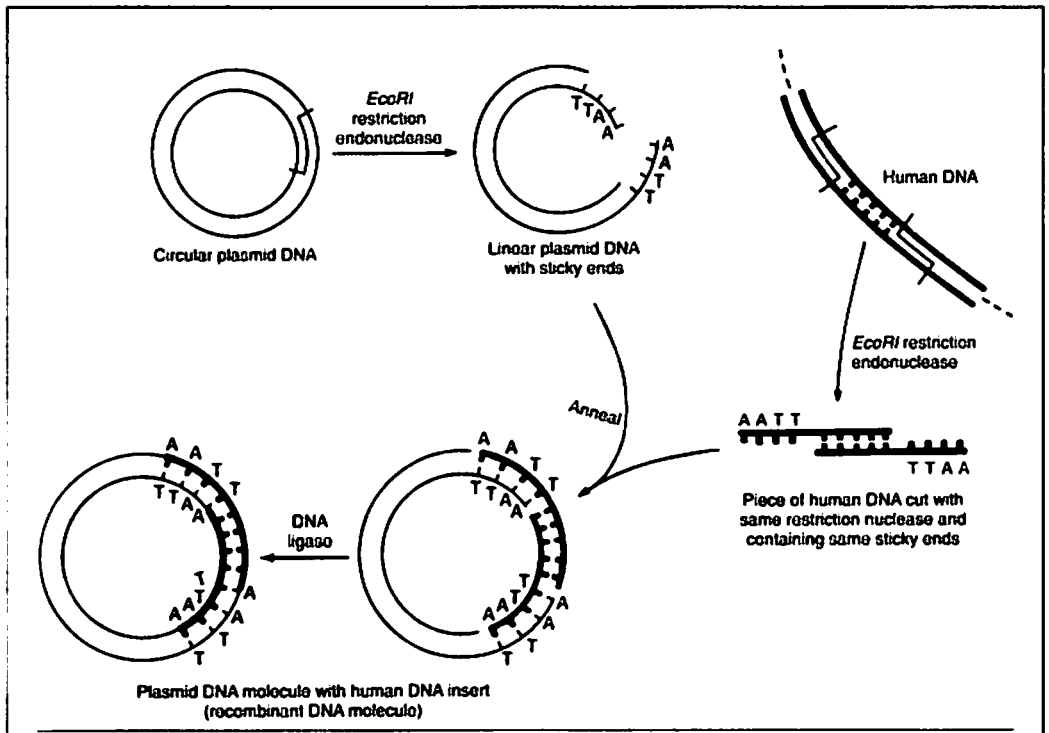


Figure 8.58: Use of restriction enzymes endonuclease to make new recombinant or chimeric DNA molecule.

b) **blunt ends:** Sometimes the previous procedure fails due to the sticky ends of the vector may reconnect with themselves, with no net gain of recombinant DNA. To overcome this problem, a restriction enzyme that generate **blunt ends** is used, and new ends are added in the form of poly d(A) tails to the 3`ends of the vector and poly d(T) to the 3` ends of the donor DNA . Thus the 2 molecules can only anneal to each other. This procedure is called **homopolymer tailing** (figure 8.59).

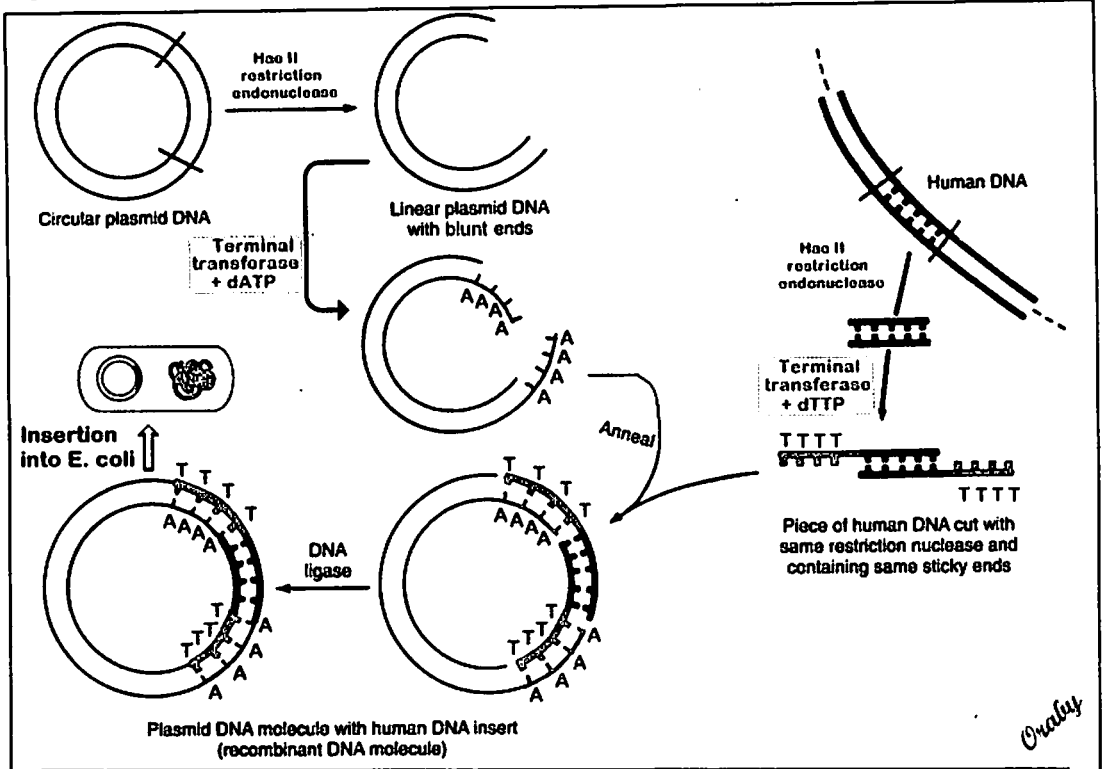


Figure 8.59: Integration of donor DNA with vector DNA, using the homopolymer tailing procedure.

III. ***Cloning of recombinant (chimeric) DNA:*** The plasmid containing the donor DNA is put into bacterial cells by a process called transformation, which then continue to replicate. One type of bacterial cells that could be used is Escherichia coli. In this way, the chimeric DNA is amplified.

A. **Clone:** Are a large number of identical molecules, bacteria or cells arising from a common ancestor.

B. **A DNA clone** is a typical copies of the original DNA.

C. **DNA cloning** is the production of a large number of identical DNA molecules arising from a common ancestor.

IV. *Importance of recombinant DNA techniques:*

A. Production of **proteins** for treatment, research and diagnosis.

B. Productions of **genes** of interest for researches and other purposes e.g. insulin gene.

C. **Gene mapping.**

D. **Gene therapy.**

E. **Molecular analysis of diseases** (pedigree analysis).

F. **Diagnosis of fetal genetic disorders.**

G. Detection of **polymorphism.**

For details, see biochemical importance of recombinant DNA technology at the end of this chapter.

In vitro techniques for DNA amplification

Polymerase chain reaction (PCR)

I. *Definition:*

A. ***It is an in vitro technique for DNA amplification.***

B. It is used to amplify a small piece of viral DNA *in* a clinical sample (e.g. plasma) up to a million folds *in test tubes* ⟹ This allows detection of small quantities of infectious agent.

C. RNA segments can also be amplified by converting them first into complementary DNA (cDNA) by reverse transcriptase (RT) enzyme:

RNA → cDNA → PCR

D. This technique was developed in 1985.

II. *Requirements for PCR:*

A. A piece of DNA template (needed to be amplified).

B. The four nucleotide bases dATP, dTTP, dGTP and dCTP (large quantities).

C. A primer sequence (large quantities), It is small pieces of DNA, 15-30 nucleotides. It binds to DNA at specific region.

D. DNA polymerase enzyme.

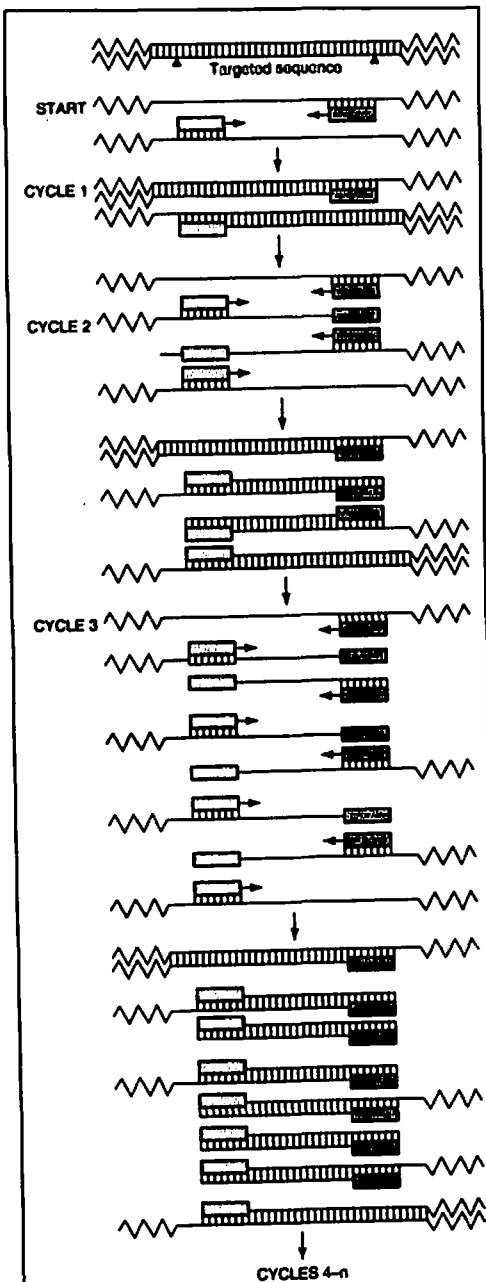


Figure 8.60: PCR

III. *Steps of PCR:*

A PCR technique is carried out in repeated 3 steps: denaturation, annealing and extenstion:

A. Denaturation:

Double stranded DNA present in the sample is heated to separate it into individual strands.

B. Annealing:

Two distinct primers are allowed to bind with the separated DNA strands. They are directed at specific sequences on opposite strands, so that they can define the segment to be amplified.

C. Extension:

DNA polymerase extends the primers in each direction and synthesizes 2 strands complementary to the original two strands.

1. This cycle consumes about 2 minutes.
2. This cycle is repeated several times (by heat denaturation, annealing the primers to their complementary sequences and extension of the annealed primers with DNA polymerase). This gives amplified product of certain length and sequence about 1 million copies. (Figure 8.60).

IV. *Uses of PCR:*

A. Detection of infectious agents e.g. HIV (AIDS) virus, and hepatitis (B and C) viruses, and bacterial bacilli of tuberculosis.

B. Diagnosis of prenatal genetic diseases. Sample is obtained by amniocentesis (amniotic fluid by trans-abdominal needle).

C. Forensic medicine: PCR allows the DNA in a single cell, hair follicle or sperm to be amplified and analyzed. Thus the application of PCR in **crimes and rape** is clear.

D. PCR is important to establish precise **tissue types** for transplants.

E. Archeological studies: PCR allows the study of **evolution** by using DNA from archeological samples.

F. PCR is used to detect **allelic polymorphism.**

Separation of DNA (or RNA) Into Genes

I. *Introduction:*

By using restriction enzymes, the entire genome can be separated into DNA fragments.

A. These fragments are inserted into a various cloning vectors.

B. This allows the entire genome to be packed into vectors.

II. *Library:*

A. It is the package of the entire genome into vectors.

B. Library can be obtained by **combining**:

1. Entire genome.
2. Restriction enzymes.
3. Various cloning vectors.

- Such combination leads to separation of DNA into fragments, which allowed to be inserted and packed into vectors ➔ Library.

C. **Types of libraries** may be genomic or complementary:

1. **Genomic library:** (contains introns and exons):
   a) **Definition:** A collection of cloned fragments that represents the entire genome. It includes both introns and exons.
   b) **Preparation:** Genomic libraries are prepared by performing partial digestion of total DNA with a restriction enzymes that cut DNA into large fragments, so that most genes will be left intact. A human library that contains million of recombinant fragments of large size has 99 % probability of being complete. Thus, the chances of finding any single copy gene are excellent.
2. **Complementary DNA (cDNA) library: (contains exons only):**
   a) **Definition:** A collection of cloned fragments that represents only the exons (or represents the population of mRNA in a cell).
   b) **Preparation:** cDNA libraries are prepared by:
      1) Isolation of all mRNAs in a tissue.
      2) **Synthesis of complementary DNA (cDNA):** Incubation of mRNAs with reverse transcriptase enzyme, different nucleotides (dATP, dGTP, dTTP and dCTP) and DNA polymerase. This leads to synthesis of DNA complementary to mRNA (see DNA replication).
      3) cDNA is then inserted into vectors .
   c) **Expression vector:** It is the vector, usually plasmid that carries cDNA that finally leads to synthesis of protein.

III. *Techniques used for detection of genes:*

These include blotting techniques, in-situ hybridization and FISH techniques.

Blotting techniques

These are molecular techniques used for **detection of specific DNA or RNA fragments** (genes) among the many thousands molecules. They are 3 types: southern, northern and western techniques.

A. **Southern blotting:** at the level of DNA (gene).
B. **Northern blotting:** at the level of mRNA (gene copy).
C. **Western blotting:** at the level of protein (gene product).

A. Southern blotting technique:

1. **Cleavage: The DNA is extracted from the cell and subjected to the restriction enzymes to be splitted into numerous smaller fragments (restriction fragments).**
2. **Electrophoresis: These DNA fragments are then subjected to agarose gel electrophoresis where different fragments are separated according to their molecular weight and charges i.e. smaller molecular weight fragments will move faster away from large molecular weight fragments.**
3. **Blotting: Then special membrane (nylon membranes) is applied to the gels where the bands (fragments) will be transferred (blotted) by capillarity from the gel to the nylon membrane.**
4. **Hybridization: Special radioactive labeled probes having complementary bases to the specific gene searched for, are then applied to the membrane where they will combine with the gene ➔ Gene detection.**
5. **Autoradiography: It is the detection of radioactive molecules (e.g. DNA, RNA and protein) by visualization of their effects on photographic films.**

6. Probes:

a) **Definition: is a single strand of DNA that can hybridize (base pair) with a complementary sequence on another single stranded DNA or RNA.**

b) **The probe must contain a label so that it can detect complementary DNA or RNA. The label may be radio active material (e.g. radioactive phosphorus, ^{32}P), so it can be detected by autoradiography or chemical that can be identified, for example by fluorescence**

c) **Uses of probes:**

1) Searching of specific gene.

2) Quantitating DNA or RNA separated by electrophoresis.

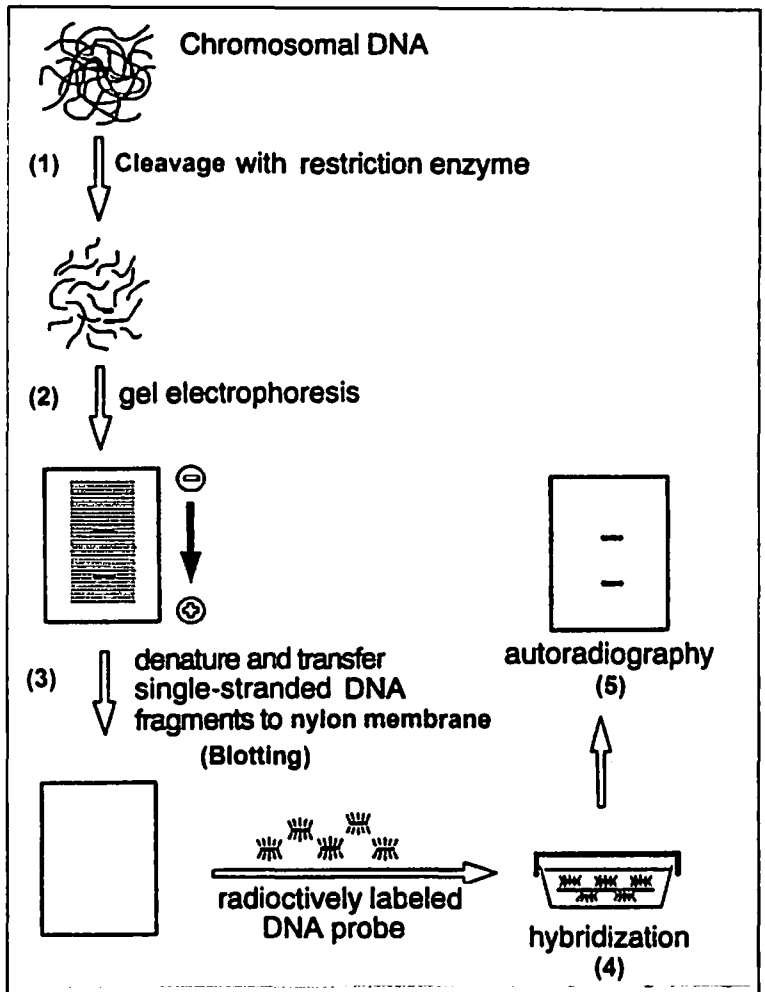


Figure 8.61: Southern blotting.

B. Northern blotting technique:

This is done by the same technique as in southern blotting except mRNA is used in replaced of DNA.

C. Western blotting (immunoblot) technique:

This is for detection of gene product (protein).

1. This is done by the same technique as in southern blotting except specific monoclonal antibody is used instead of the probe to detect the protein product of the gene in request.

2. HIV virus is diagnosed by Western blotting technique. It detects the protein of the virus.

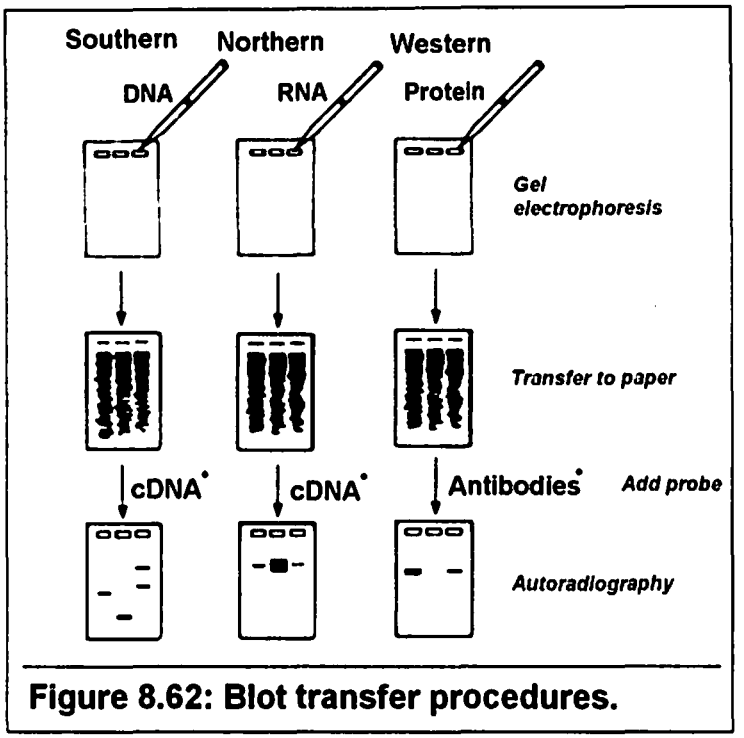


Figure 8.62: Blot transfer procedures.

D. **Uses of blotting techniques:**

1. Diagnosis of infectious diseases as AIDs.
2. Diagnosis of malignant diseases.
3. Diagnosis of genetic diseases.
4. Used in forensic medicine e.g. paternity.
5. Human leucocytic antigen (HLA) typing for organ transplantation (HLA are antigen present on the surface of leucocytes).

In-situ Hybridization (ISH)

When a probe is added to tissue section on a glass slide, hybridization occurs by binding between bases of probe and DNA. This is called in-situ hybridization.

Fluorescence In-situ hybridization (FISH)

I. When the probe is used for detection of specific gene or chromosome is labeled by fluorescent material, the hybridization will be detected by

the obtained fluorescence, this is known as **Fluorescence In-situ hybridization (FISH):**

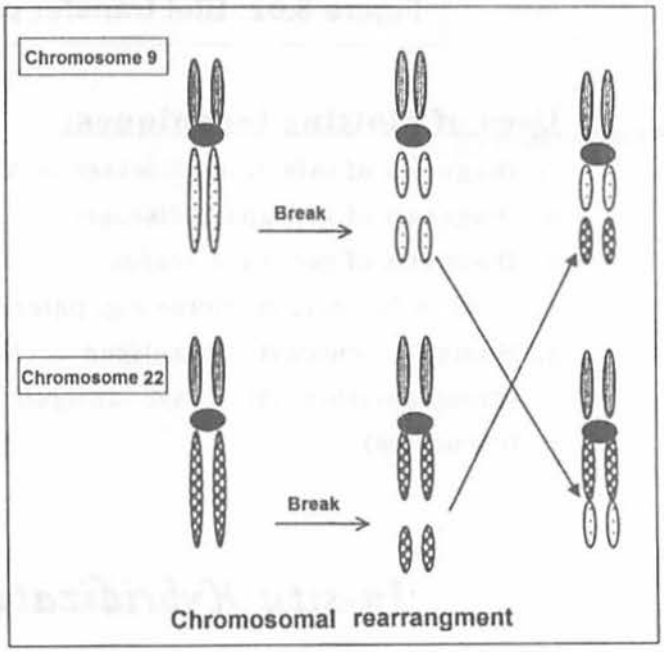
The FISH technique is an important molecular biology and molecular genetic technique that can be used for detection of:

Down syndrome.

A. **Numerical variation in chromosomes:**

Normally each chromosome is represented within a cell by 2 copies, so when we prepare a red fluorescent probe and added to a mixture of chromosomes, it will give 2-red fluorescence. This is **diploid** or **normal pattern** of the chromosomes. On the other hand 3-red fluorescence indicates **trisomy** (an extra chromosome) as in **Down syndrome** and only one red fluorescence indicates **monosomy** (absence of one copy)

B. **Structural variation of chromosomes e.g. translocation:**



This can be explained by the following example: when chromosome **number 9 is labeled** red and **chromosome number 22 is labeled green,** so in each stained normal cell there will be 2 red and 2 green spots. When we detect a spot that contains both green and red fluorescence. This means chromosomal translocation as in **Philadelphia chromosome (9,22)** as in **chronic myeloid leukemia** where a part of chromosome number 9 (red) is transferred (translocated) to chromosome number 22 (green), so there will be red and green colors at the point of translocation.

Techniques for identifying DNA Sequences (DNA Sequencing)

I. Definitions:

A. DNA sequencing is the reading and monitoring of the individual bases that constitute a gene or genes.

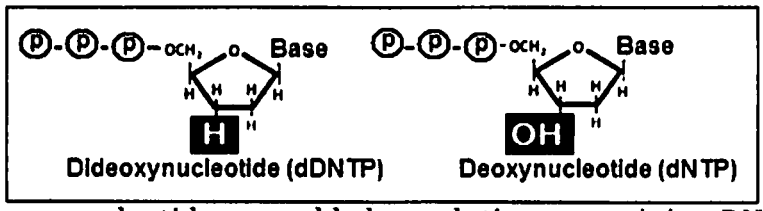
B. Knowing the sequence of bases along a gene enables the synthesis of **specific probes** used for detection of their genes.

C. DNA sequencing leads to detection and localization of various genes among the entire genome. **(=human genome project)**.

II. Techniques for identifying DNA Sequences:

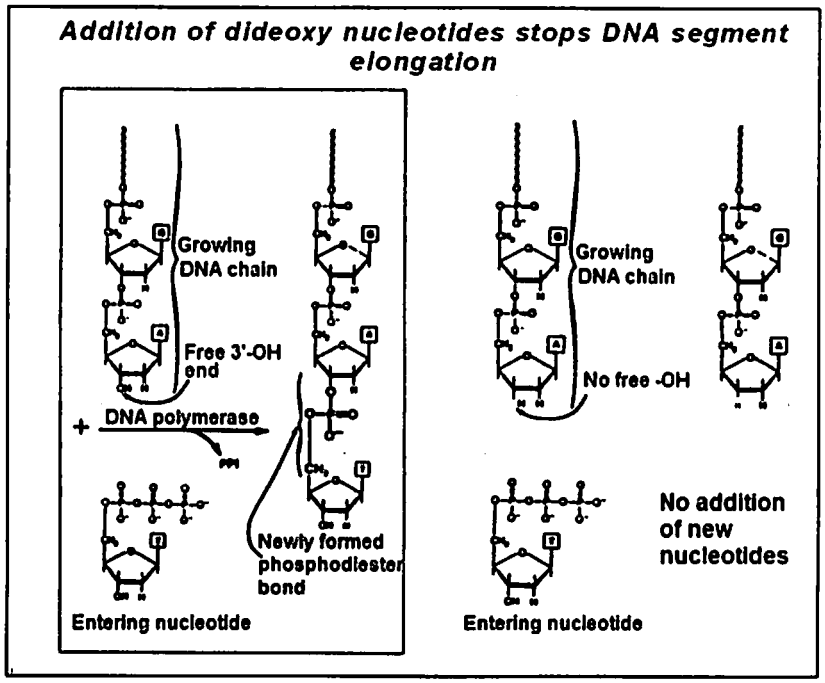
A. Manual, enzymatic method (Sanger's method):

1. By using dideoxynucleotides: ddATP, ddGTP, ddCTP, ddTTP.

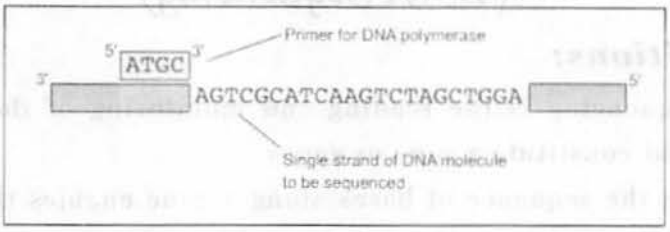


2. Dideoxynucleotides are added to solutions containing DNA chain and DNA polymerase.

3. Because dideoxynucleotides do not contain a 3'-hydroxyl group, they terminate DNA chain elongation at specific nucleotides as they cannot form a phosphodiester bond with the next deoxynucleotide.

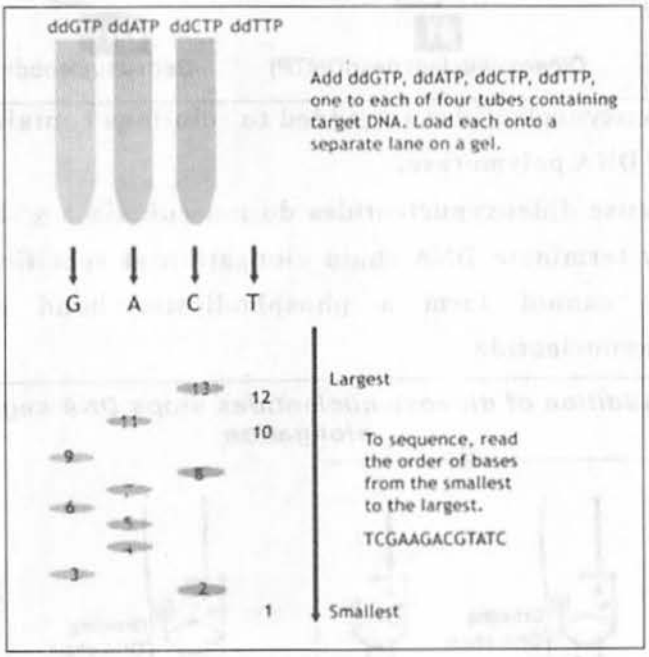


4. Before the DNA can be sequenced, it has to be denatured into single strands using heat.
5. Next, a primer is annealed to one of the template strands.



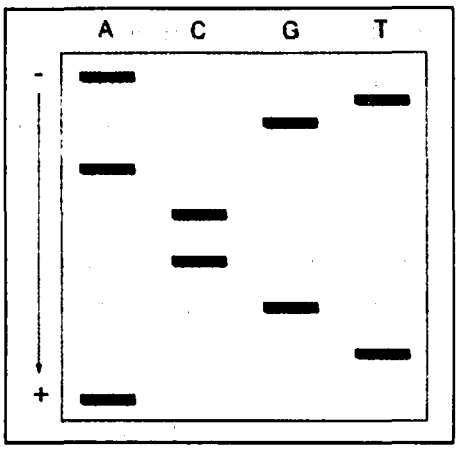
6. Once the primer is attached to the DNA, the solution is divided into four tubes labeled "G", "A", "T" and "C". Then reagents are added to these samples as follows:

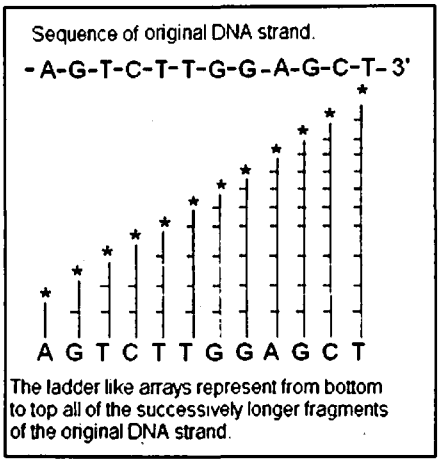
G" **tube:**	all four dNTP's, **ddGTP** and DNA polymerase
A" **tube:**	all four dNTP's, **ddATP** and DNA polymerase
T" **tube:**	all four dNTP's, **ddTTP** and DNA polymerase
C" **tube:**	all four dNTP's, **ddCTP** and DNA polymerase



7. As the DNA is synthesized, nucleotides are added on to the growing chain by the DNA polymerase.
8. However, and because the dideoxynucleotides compete with normal nucleotides for incorporation into the growing chain, the dideoxynucleotide is occasionally incorporated into the chain in place of a normal nucleotide, which results in a chain-terminating event. A **DNA chains of varying lengths are produced.**

9. The shortest chains are nearest the 5′ end of the DNA chain (which grows 5′ to 3′).
10. The sequence of the growing chain can be read (grows 5′ to 3′) from the bottom to the top of the gel on which the DNA chains are separated.





B. Automated method of DNA sequencing:

1. **The automated method of DNA sequencing is now a routine laboratory procedure. All reactions are performed in a single tube and the reactions are terminated using fluorescent dideoxynucleotides.**

Summary of molecular biology techniques:

Technique	Function
DNA amplification:	
1-Cloning	**In vivo DNA amplification**
2-PCR	**In vitro DNA amplification**
Detection of genes:	
1- Blotting techniques:	
• Southern blotting	**DNA (genes) detection.**
• Northern blotting	**RNA gene copy) detection**
• Western blotting	**Protein (gene product) detection**
2- In-situ hybridization (ISH)	**Detection of DNA on a slide by a microscope**
3- FISH	**Detection of DNA by fluorescence microscope**
DNA sequencing:	
Manual method (Sanger's)	**Localization of site and sequence of bases (i.e. genes) along DNA.**
Automated	
RFLP	**Detection of DNA polymorphism**

Biochemical importance of recombinant DNA technology

I. ***Gene mapping*:** It means construction of map of human genome. i.e. localization of specific genes in distinct chromosomes.

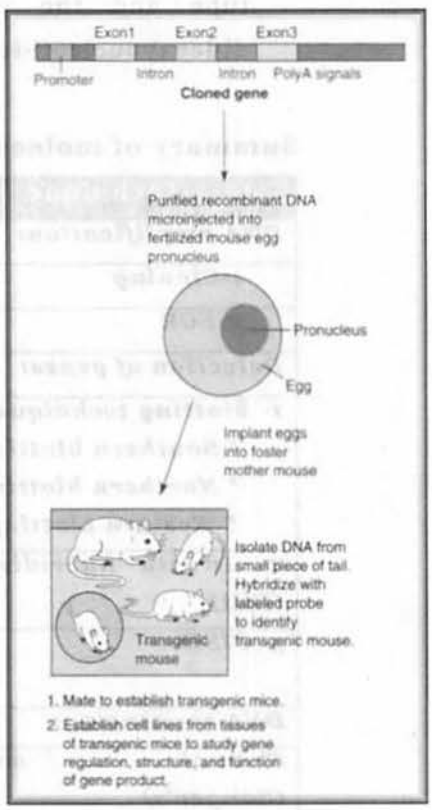
A. Gene mapping gives useful information about human diseases.

B. **ISH** (in situ hybridization) and **FISH** (fluorescence in situ hybridization) are 2 techniques used in gene mapping.

1. Somatic cell chromosomes are hybridized with specific radioactive probe. By this way, the exact area of hybridization is localized and we can know the exact place of the gene on the chromosome.
2. By using other radioactive probes, one after another, other gene localization can be identified. This leads to a construction of a gene map.

II. ***Production of proteins for treatment, research and diagnosis:*** For example, human insulin hormone is produced for treatment of diabetes mellitus. Also hepatitis B vaccine is produced for prevention of hepatitis B disease.

III. ***Gene therapy:*** Diseases caused by deficiency of a gene product (e.g. sickle cell anemia) are aimed to cure through gene therapy. Once the **"bad"** genes are identified; there will be challenge to cure them through gene replacement.



A. The strategy is to clone a desired gene into a vector that will be taken up and incorporated into the genome of the host cell.

B. **There are 2 ways of introducing a gene into the host cell :**

1. **Transgenic animals**: by transferring it into germ cells such as sperm, egg or fertilized ovum. The new gene would be passed on to the next generations. This has been tested only in **experimental animals** and the hundreds of offspring are called **transgenic animals.**

2. By transferring new gene into somatic tissues like bone marrow mother cells. This can be used in treatment of leukemia.

IV. ***Molecular analysis of diseases (pedigree analysis):***

To understand diseases at a molecular level e.g. sickle cell anemia.

V. ***Diagnosis of fetal genetic disorders:*** DNA from cells collected from amniotic fluid or from chorionic villi can be analyzed by southern blot transfer procedure. This leads to diagnosis of genetic disorders of the fetus before delivery e.g. sickle cell anemia and phenylketonuria.

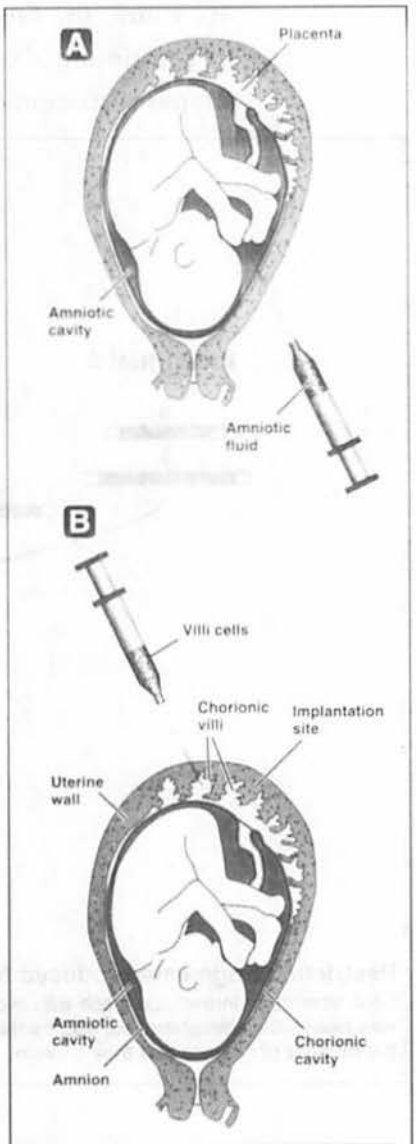
VI. ***Detection of polymorphism:*** **by restriction fragment length polymorphism (RFLP):**

A. Polymorphism is a variation in nucleotide sequence from one individual to another.

B. **Human differ in their composition. Polymorphisms (variation in DNA sequences) occur frequently in the genome both in coding (exons) and non-coding (introns).** Point mutations cause the simplest type of polymorphisms, but insertions and deletions of varying lengths also occur.

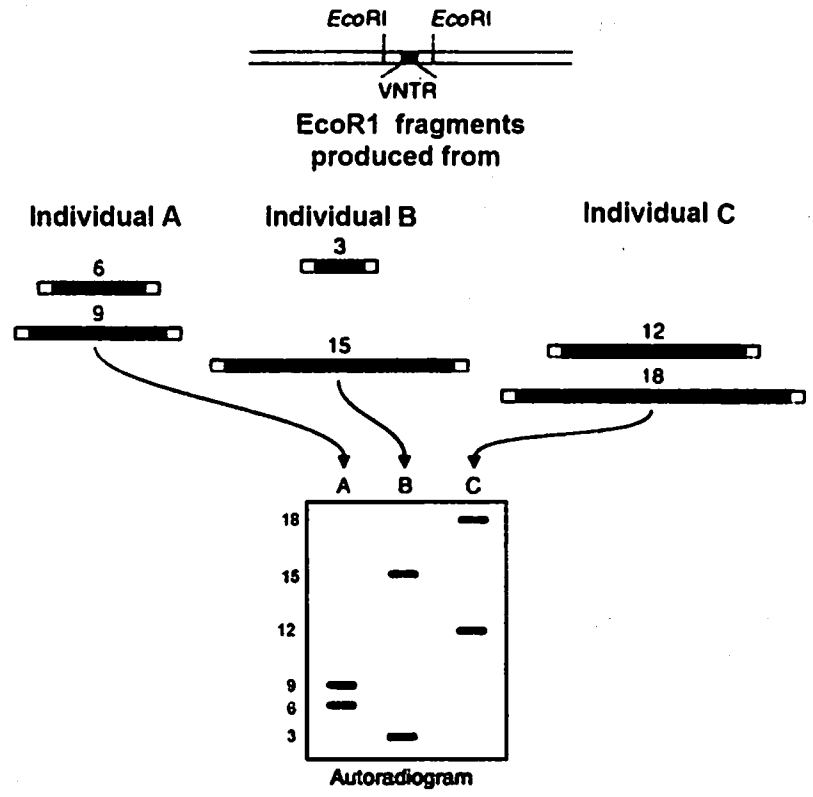
C. **Restriction fragments length polymorphism (RFLP):**

1. **Occasionally, a mutation** occurs in **a restriction enzyme cleavage site** that is within or tightly linked to a gene. The enzyme can cleave the normal DNA at this site, but not the mutant. Thus, 2 smaller restriction fragments will be obtained from this region of the normal DNA, compared with only one larger fragment from the mutant.



Sampling of fetal cells:
A: Amniotic fluid; B: Chrionic villus.

2. Normal human DNA has many regions that contain a highly variable number of tandem repeats **(VNTR)**. The number of repeats ranges from 50,000 to 100,000 times in the human genome and differs from one individual to another (and from one allele to another). Restriction enzymes that cleave on the left and right flanks of a **VNTR** produce **DNA fragments of variable length.** The length depends on the number of repeats that the DNA contains.
3. Fragments produced by various restriction enzymes from a number of different loci can be used to identify individuals with the accuracy of fingerprints. Therefore, a technique called "DNA fingerprinting" is used in forensic medicine as to implicate suspects in criminal cases.



Restriction fragments produced from a gene with a variable number of tandem repeats(VNTR). DNA from three individuals, each with two alleles for this gene and a different number of repeats in each allele, was cleaved, electrophoresed, and treated with a probe for this gene. The length of the fragments depends on the number of repeats that they contain.

Human Genome Project

I. Introduction:

A. **Human Genome Project**, which started in 1990, is an international effort whose principle goals were to sequence the entire human genome and the genome of several other model organisms e.g. E. coli.

B. This genetic information is found in each cell of the body, encoded in the chemical deoxyribonucleic acid (DNA). Through a process known as sequencing.

C. Although the completion of the Human Genome Project was celebrated in April 2003 and sequencing of the human chromosomes is essentially "finished," the exact number of genes encoded by the genome is still unknown.

D. The Human Genome Project has identified nearly all of the estimated **20,000 to 25,000 genes** (the basic units of heredity) in the nucleus of a human cell.

E. The project has also mapped the location of these genes on the 23 pairs of human chromosomes, the structures containing the genes in the cell's nucleus.

II. Goals:

A. **Determine all 3 billion base pairs** in the human genome with a minimal error rate

B. **To identify all the genes in the human genome**. This part of the project is still ongoing as about 30,000 genes in the human genome are only detected, which is far fewer than predicted by most scientists.

C. To develop faster and more efficient methods for **DNA sequencing** and sequence analysis and the transfer of these technologies to industry.

D. The sequence of the human DNA is stored in database known as **Genbank** is available to anyone on the Internet.

E. The process of identifying the **boundaries between genes** and other features in raw DNA sequence is called genome annotation and is the domain of bioinformatics.

F. All humans have unique gene sequences; The Human Genome Project genome aims in identifying differences between individuals.

Most of the current effort in identifying differences between individuals involves single nucleotide polymorphisms.

III. ***Benefits***

A. **Diagnosis of genetic diseases** e.g. breast cancer, blood clotting, cystic fibrosis, liver diseases and many others.

B. There are also many **benefits for biological scientists.** For example, a researcher investigating a certain form of cancer may have performed his search to a particular gene. By visiting the human genome database on the worldwide web, this researcher can examine what other scientists have written about this gene, The list of data types explains why bioinformatics is so challenging.

C. The work on interpretation of genome data will help in the fields of **medicine and biotechnology,** leading to cures for cancer, Alzheimer's disease and other diseases.

D. On a more philosophical level, the analysis of similarities between DNA sequences from different organisms is opening new avenues in the study of the **theory of evolution.**

E. The DNA analyzed in the Human Genome Project came from small samples of blood or tissue obtained from many different people. Although the genes in each person's genome are made up of unique DNA sequences, the average variation in the genomes of two different people is estimated to be 0.05 to 0.1 percent. That is, approximately 1 in 1,000 to 1 in 2,000 nucleotides will be different from one individual to another. Thus the differences between human DNA samples from various sources are small in comparison to their similarities.

IV. ***Mapping and Sequencing:*** There are two main categories of gene-mapping techniques:

A. **linkage, or genetic, mapping,** a method that identifies only the relative order of genes along a chromosome.

B. **physical mapping,** more precise methods that can place genes at specific distances from one another on a chromosome.

C. Both types of mapping use markers in the DNA sequence, detectable physical or molecular characteristics that differ among individuals and that are passed from one generation to the next.

V. ***Bioinformatics***

A. The completed human genome sequence generated a catalog made up of around 20,000 to 25,000 human genes; high-resolution maps of the chromosomes, including hundreds of thousands of landmarks; and

billions of base pairs of DNA-sequence information. Laboratory information-management systems, robotics, database-management systems, and graphical user interfaces were among the computing tools required to help genome researchers make sense of this flood of data.

B. A new field of research, bioinformatics, has developed in part to address the computing challenges raised by the project. Researchers in bioinformatics have developed public databases connected to the Internet to make genome data available to scientists worldwide, along with analytical software for making sense of this flood of biological information. For example, DNA-sequence information is stored in several databases, including the NIH's GenBank, the European Molecular Biology Laboratory's Nucleotide Sequence Database, and the DNA Databank of Japan.

VI. ***Project Status***

A. In February 2001 a rough draft of the DNA sequence of the human genome was published. The draft provided a basic outline of 90 percent of the human genome. Researchers produced a finalized version of the complete sequence of the human genome in April 2003, two years earlier than originally projected.

B. With the completion of the human genome, scientists now have a more detailed blueprint of the human genetic code. Scientists were surprised to learn that the actual number of human genes is far lower than expected—only about 20,000 to 25,000 genes compared to the predicted 100,000 genes. This number is a little more than twice the number of genes found in the fruit fly.

C. In addition to working on the human genome, researchers have sequenced the complete genome of a number of organisms—many bacteria, including *Escherichia coli*; the yeast *Saccharomyces cerevisiae*; the roundworm *Caenorhabditis elegans*; and the fruit fly. This research helps scientists find parallels between human genes and the genes of other life forms and thus helps them better understand the biological functions of the genes.

D. With the human genome sequence completed, scientists are now focusing their attention on the proteins encoded by human genes. In the relatively new science known as proteomics, scientists seek to detail the function of all human proteins. This information may lead to the development of new drugs for treating many human disorders. Proteomics may eventually lead to more novel ways to correct fatal flaws in the human genetic heritage, dramatically changing the medical approach to disease.

E. Increased knowledge of the human genome also has many controversial ethical, legal, and social implications. The project's findings to date have already sparked worldwide debate on the ethics and legality of patenting human gene sequences for commercial use, the possibility that private genetic information will become available to insurance companies and employers, and the potential danger of correcting genetic defects in ways that would be passed from one generation to the next.

VII. ***Whose genome was sequenced?***

A. The human genome reference sequences do not represent any one person's genome. Rather, they serve as a starting point for broad comparisons across humanity. The knowledge obtained is applicable to everyone because all humans share the same basic set of genes and genomic regulatory regions that control the development and maintenance of their biological structures and processes.

B. In the international public-sector Human Genome Project (HGP), researchers collected blood (female) or sperm (male) samples from a large number of donors. Only a few of many collected samples were processed as DNA resources. Thus the donor identities were protected so neither donors nor scientists could know whose DNA was sequenced. DNA clones from many different libraries were used in the overall project.

C. Technically, it is much easier to prepare DNA cleanly from sperm than from other cell types because of the much higher ratio of DNA to protein in sperm and the much smaller volume in which purifications can be done. Using sperm does provide all chromosomes for study, including equal numbers of sperm with the X (female) or Y (male) sex chromosomes. However, Human Genome Project scientists also used white cells from the blood of female donors so as to include female-originated samples.

D. Many small regions of DNA that vary among individuals (called polymorphisms) also were identified during the Human Genome Project, mostly single nucleotide polymorphisms (SNPs). Most single nucleotide polymorphisms are without physiological effect, A much smaller minority of polymorphisms affect an individual's susceptibility to disease and response to medical treatments.

E. Although the HGP has been completed, single nucleotide polymorphisms studies continue in the International Haplotype Map Project, whose goal is to identify patterns of single nucleotide polymorphisms groups (called haplotypes, or "haps"). The DNA samples for the Haplotype Map came from a total of 270 individuals.

دعاء

اللهم إني أسألك خير المسألة وخير الدعاء

وخير النجاح وخير العلم وخير العمل

وخير الثواب وخير الحياة وخير الممات

وثبتني وثقل موازيني وحقق إيماني

وارفع درجتي وتقبل صلاتي واغفر خطيئاتي

وأسألك العلا من الجنة

اللهم احرسني بعينك التي لا تنام

واكنفني بركنك الذي لا يرام

وأحفظني بعزك الذي لايضام

واكلأني في الليل والنهار

وارحمني بقدرتك علي

أنت ثقتي ورجائي

فيا من قل عند نعمته شكري فلم يحرمني

ويا من قل عند بلائه صبري فلم يخذلني

ويا من رآني علي الخطايا فلم يعاقبني

يا ذا المعروف الذي لا ينقضي أبدا

وياذا الأيادي التي لا تحصي عددا

ويا ذا الوجه الذي لا يبلي أبدا

ويا ذا النور الذي لا يطفأ سرمدا

يا حي ياقيوم برحمتك أستغيث

أصلح لي شأني كله

ولا تكلني إلي نفسي طرفة عين

رقم الإيداع ١٤٤٨٩ / ٢٠٠٥

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